EFFECTS OF ZINC METABOLISM UPON GUSTATORY SENSITIVITY

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ABSTRACT

A series of experiments was conducted to investigate the relationships between zinc metabolism and gustatory sensitivity. Among hospitalized alcoholic subjects, there was an incidence of decreased taste sensitivity, both subjectively and objectively. These subjects had lower than normal plasma zinc concentration levels at the time of admission to the hospital. Without medication, there were no significant changes in taste perception of these subjects during the first two or three weeks of hospitalization. In a single-blind study of 25 alcoholic subjects, both the subjects treated with zinc sulphate at dosages of 150 mg Zn^{++} daily and those treated with placebos showed significant improvements in taste sensitivity to sweet, salt and bitter stimuli but not to sour stimuli during the three weeks of therapy. There were no significant differences in taste responses between the subjects in the zinc treatment and placebo groups. An exploratory study into the effects of zinc sulphate solutions, used as mouthrinses, on taste perception was also carried out; the results showed that zinc sulphate had no significant effect on the perception of sweetness of sucrose. The overall results were interpreted as contradictory to the hypothesis that zinc metabolism played a significant role in taste processes. The results were discussed in the light of previous work by Henkin and his associates, which had suggested that zinc played a crucial role in taste acuity.

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CHAPTER I

INTRODUCTION

Changes in gustatory sensitivity due to different factors have been noted in the past, but the causes of these changes other than the physical properties of the stimulus or the environment e.g. temperature of the tastant solutions, atmospheric pressure of the places where the sensitivity is assessed, or even the wearing of dentures, have not, until recently been thoroughly investigated. Barycheva as early as 1936 concluded that fluctuations in the thresholds of taste could occur in anyone owing to various factors "acting on the nervous system". He suggested that sensitivity could be enhanced or depressed.

Up to the mid 1960's, most of the interest in "Taste Blindness" was directed towards differences in reactions to some chemical stimuli, the most popular being the substances p-ethoxyphenylthiourea and phenylthiocarbamide, both of which are bitter to some people and tasteless to others, at some concentrations. The results from these studies led to suggestions that taste deficiency is genetic and in accordance with Mendel's Laws (Snyder 1931, Fox 1932). This suggestion however, has been disputed again and again and remains unconfirmed at the present time.

Jacobs (1955) worked in the field of taste variability and considered the possibility of classifying individuals into taste groups using the chemical sodium benzoate which produced variable taste responses including bitter-sweet, bitter-sour, bitter and tasteless among different subjects. He concluded that individuals might show varied responses to taste chemicals but

that classification into taste groups was not feasible.

Other workers in this area used the notation of four primary taste sensations to investigate taste deficiencies. They assumed that there must be some individuals who are deficient in one or more of these primary taste sensations while having normal sensations to other taste primaries. Moncrieff (1967) noted that no such case has been reported. He wrote:

> "No person is known, for example, to be sensitive to sour, salt and sweet, but insensitive to bitter substances. True, taste may be completely lost by damage to the nervous system, but no destruction of one taste, leaving the others unimpaired has been noted."

This statement, however was later refuted because cases have been reported where alterations in taste threshold in some and not other sensations were exhibited. These results were from studies of taste threshold levels among some patients showing different disease states, as follows.

After more than a decade of observations and researches, a group of investigators at the National Heart and Lung Institute, Bethesda, Maryland U.S.A, demonstrated that taste alterations occur in several disease states. For example, patients with gonadal dysgenesis (Henkin 1967) and pseudo-hypoparathyroidism (Henkin 1968) exhibited elevated thresholds for detection and recognition of the taste of sour and bitter, while patients with Cushing's syndrome (Henkin 1969) and familial dysautonomia (Henkin and Kopin 1964) exhibited elevated thresholds for all four taste qualities.

Further, this group of researchers initiated a new approach to the study of "Taste Blindness". They investigated the changes in gustatory sensitivity due to the changes in bodily metabolism of the patients concerned and the methods of treating these abnormalities in taste sensations.

Apart from the group of patients with relatively rare

diseases mentioned above, these researchers have also described an "unexpected large group with decreased taste acuity (hypogeusia) without any obvious underlying cause". They also reported that besides hypogeusia, these patients often complain that many foods have an abhorrent or inappropriate taste (dysgeusia), that they have decreased smell acuity (hyposmia), and that many odorants have an abhorrent or inappropriate smell (dysosmia). Henkin et al. (1971) called this syndrome "Idiopathic hypogeusia with dysgeusia, hyposmia and dysosmia".

The treatment of this syndrome, Henkin and his associates suggest, can be achieved with success by the administration of zinc in the form of zinc sulphate. This suggestion was derived from experiments done with both humans and animals wherein experimentally induced changes in trace metals or in thiol concentrations can alter taste acuity (Henkin and Bradley 1969). The oral administration of zinc ion in doses of 100 mg/day in a single blind study resulted in a significant improvement in taste acuity among patients with idiopathic hypogeusia (Schechter et al. 1972). This result suggests a relationship between zinc metabolism and gustatory sensitivity.

As these investigators themselves pointed out (Schechter et al. 1972), at this stage, it is not possible to predict which patients will improve on zinc therapy. No correlation can yet be made between the etiology of the syndrome, duration of the symptoms, initial serum and urinary metal concentrations and the effectiveness of zinc therapy.

So far, investigations had only been conducted on patients with rare diseases. The patients suffering from the newly described syndrome, idiopathic hypogeusia with dysgeusia, hyposmia and dysosmia are only reported by this group of researchers and

are very seldom seen in a clinical setting. The alterations in taste functioning of these patients are very severe and the patients themselves seek treatment to relieve these symptoms. It is not known whether zinc medication would have the same effect among patients who complain of subjective alterations in taste functioning which, while not being so severe as to be distressful to the patients and for them to seek medical assistance, these alterations in taste functioning nevertheless cause discomfort and lead to reduction in appetite, commonly seen in hospitalised patients with different ailments.

It is the aim of this research to investigate further into the relationship between zinc metabolism and gustatory sensitivity.

It is well known among those who work in hospitals which provide treatment for alcoholism that chronic alcoholic patients often present themselves for admission to the hospitals in nutritionally deficient states. This is due to the lack of a balanced diet and prolonged alcoholic intake. It has also been shown that the majority of hospitalised alcoholic patients, both with and without symptoms of liver dysfunctions, exhibit zinc deficiency in their bodies at the time of admission into the hospital (Sullivan and Lankford 1962, 1965).

Many chronic alcoholic patients complain of subjective decrease in taste sensitivity. When asked about their eating habits, these patients report that all foods taste the same to them and that they cannot appreciate different flavours to the same extent as they had been able to prior to prolonged alcoholic intake. Smith (1972) reported objective increase in taste thresholds to quinine among alcoholic patients.

If the hypothesis of Henkin and his colleagues, which suggests a relationship between zinc metabolism and gustatory

sensitivity, is applicable in all cases of abnormal taste sensitivity, the apparent decrease in taste sensitivity among alcoholic patients may be interpreted as the result of deficiency in the zinc metabolism of these patients. When treatment with zinc medication, which corrects zinc deficiency, is administered, we may expect significant improvements in taste sensitivity among these patients. A series of experiments has been conducted to investigate into the relationship between taste sensitivity and zinc metabolism among hospitalised alcoholic patients.

Zinc medications have been used successfully in the treatment of chronic leg ulcers, where it has been found that zinc was effective both as orally administered zinc sulphate and as compounds in ointments to be topically applied to the wounds. Since the oral administration of zinc sulphate has been shown to be effective in the treatment of taste abnormalities, it is of interest to study whether zinc sulphate has any significant effect on taste perception when it is applied topically as a mouthrinse.

This thesis, therefore, contains a report of the results of a series of experiments which were designed to study the relationship between zinc metabolism and gustatory sensitivity among hospitalised alcoholic patients, together with the results of a preliminary investigation into the effects of zinc sulphate mouthrinses on taste perception. Before these experiments are presented, relevant background information on zinc metabolism, taste functioning and a discussion of the studies undertaken by Henkin and his associates, will be provided.

CHAPTER II

ZINC METABOLISM IN HEALTH AND DISEASE

In discussing the role of zinc therapy in various pathologic conditions, Naess (1969) suggests that there are three main reasons for the newly awakened interest in zinc.

(1) an increased understanding of the possibility that a lack of certain elements may be an important factor in the pathogenesis of several diseases,

(2) the knowledge that zinc is a constituent part of several enzymes,

(3) the fact that it is now possible to determine the zinc concentration in biological tissues and fluid with an acceptable accuracy.

The interest in this metal is further enhanced by several reports of possible connections between zinc metabolism and some disease states for example, schizophrenia (Pfeiffer and Iliev 1972) chronic alcoholism (Sullivan and Lankford 1965) and liver diseases (Halsted et al. 1968). Of particular interest to clinicians are the reports of success of treatment employing zinc salts in several disease states including Dwarfism in Egypt (Sanstead et al. 1967) and Iran (Halsted et al. 1972), wound healing (Pories, Henzel and Strain (1967), Haeger, Lanner, Magnusson (1972), among many others), thermal burns (Henzel, De Weese and Pories 1967), and Atherosclerosis (Pories et al. 1967).

Over the past two decades, information on zinc metabolism has been accumulated. Some important knowledge in this area has been achieved through studies of Zn65, the isotopic zinc metabolism. These Zn65 studies on human subjects are few in

number and its suitability in studies of turn-over rate has been questioned (Fox 1970), due to its lengthy biological half life in man. The basic relevant information on zinc metabolism will be discussed briefly here.

I. AVAILABILITY IN NATURAL FOODS

As Fox (1970) points out, in normal conditions, a severe zinc deficiency resulting from inadequate intake of zinc is unlikely to occur with a varied diet of natural foods. Zinc is widely distributed in foods, drinking water and the air. A well balanced human adult diet supplies some 10 to 15 mg of zinc daily. Most zinc salts are absorbed and are equally well utilised; even metallic zinc can be dissolved by gastric juices and become available for absorption. It is estimated (Orten 1966) that the daily requirement of zinc is about 1 to 2 mg. Zinc is primarily associated with protein foods, for example milk, meat, fish, eggs, nuts, wholegrains and legumes. Apart from cow's milk, the zinc content of foods appears to be somewhat less than half that of iron by weight and to show a parallel distribution to iron.

II. ABSORPTION

Zinc absorption, like iron absorption occurs in the small intestine, predominantly in the duodenum (Underwood 1971). Little is yet known of the mechanisms involved at the present time. Studies using isotopic zinc, Zn65, in rats (Feaster et al. 1955) and steers (Feaster et al. 1954) have shown that only some five to ten per cent of dietary zinc is absorbed at a normal intake level. In general, values obtained for the percentage of dietary zinc absorbed are similar to those for the absorption of dietary iron

(Orten 1966).

Absorption is affected by the level of intake of the element, by the amounts and proportions of several other elements and dietary components for example, the elements copper and calcium, and the ingestion of phytic acid through soy bean diets (Oberleas, Muhrer and O'Dell 1966). The chemical form in which the zinc is ingested also influences absorption rate (Underwood 1971). There is questionable evidence that zinc absorption decreases with age in rats (Gunn, Gould, and Anderson 1963) and cattle (Miller and Cragle 1965). However, there are some doubts whether this is a true maturity effect. Further evidence on factors affecting zinc absorption will be discussed later.

III. EXCRETION

The principal pathway of zinc excretion appears to be by the faeces. Fecal zinc consists mostly of unabsorbed dietary zinc with a small amount of endogeneous origin. This endogeneous zinc is secreted mainly into the small intestine, chiefly via the pancreatic juice. Little is normally secreted by way of the bile or urine (Methfessel and Spencer 1966). Results from Zn65 studies show that injected zinc is mostly excreted in the faeces with little appearing in the urine (McCance and Widdowson 1942). When the zinc is given intraveneously, 20 per cent appeared in the faeces and 0.25 per cent in the urine (Feaster et al. 1954). This pattern of excretion is observed to be followed on diets normal in zinc and on those high in zinc (Underwood 1971).

Zinc excretion in urine has been studied by some investigators recently (Vallee et al. 1957, Prasad et al. 1963). Normally, a small amount of zinc is excreted in the urine, 457 $\pm$ 120 micrograms of zinc per day is lost in urine in a normal adult.

(Vallee et al. 1957). The quantities of urinary zinc do not vary appreciably with the dietary level of zinc and are not significantly increased even when the plasma zinc level is raised following zinc administration. Urinary excretion of zinc has been studied in several disease states, the excretion is found to be well above normal in total starvation (Spencer and Samachson 1970), hypertension (Schroeder 1957), and post alcoholic cirrhosis (Prasad, Oberleas and Halsted 1965).

In a study by Prasad et al. (1963) in Egypt, the zinc content in the sweat of normal individuals was found to be 115 $\pm$ 30 micrograms per 100 ml. In cell-free sweat, the zinc content was 93 $\pm$ 26 micrograms per 100 ml. In zinc-deficient patients, the mean zinc level in the whole sweat was reduced to 60 $\pm$ 27 micrograms per 100 ml. Thus in a hot climate where Prasad et al. approximated the amount of sweat to be five or more litres per day, a normal individual can lose 5mg Zn/day and a Zn-deficient individual, about 2mg Zn/day. In temperate climates, the losses of zinc by this route are of course, substantially smaller.

IV. INTERMEDIARY METABOLISM

Absorbed or injected zinc is incorporated at different rates into different tissues which reveal varying rates of zinc turnover.

Within the intravascular compartment, zinc exists in erythrocytes - the red blood corpuscles, leukocytes - the white blood corpuscles, and as a bound complex with serum proteins, particularly albumin and globulin. During stabilized homeostasis approximately 75 per cent of whole blood zinc is contained within erythrocytes, 12 per cent to 22 per cent in plasma and 3 per cent within leukocytes. Vallee (1963) gives a figure of 120 micrograms per 100 millilitres as the average normal adult serum

zinc level, with the range taken from other workers between 95 to 160 micrograms per 100 millilitres. Foley et al. (1968) found that among fourteen normal adult subjects, zinc content of serum is consistently higher than the zinc concentration in plasma by an average of 16 per cent - they attribute this difference in zinc concentration to greater dilution in plasma (39 per cent of the increase), zinc contents of platelets (44 per cent of the increase) and hemolysis (4.0 per cent of the increase). However, many researchers in this field still use the terms serum and plasma interchangeably. This reflects the view as stated by Vikbladh (1951) that "Serum and plasma have the same zinc content". The reason behind this difference in opinion can be that Foley et al.'s report is the only evidence suggesting zinc content in blood platelets and also as Henzel et al. (1967) puts it "Qualitative biologic determinations vary according to the method of analysis and experience of the analytical chemist, as is the case for most of the elements which occur in relatively minute concentration".

Approximately one third of the zinc content in serum (and plasma) is "firmly bound" while the other two thirds is relatively "loosely bound" to protein. Alpha globulin appears to bind more zinc than any other human plasma protein fraction when expressed in terms of zinc bound per milligram of protein (Orten 1966). As yet, however, no specific plasma protein has been definitely identified as a transport protein for zinc, as is true in the case of iron (transferrin) and of copper (ceruloplasmin). Zinc is present in erythrocytes primarily as the enzyme carbonic anhydrase and in leukocytes as a protein complex, non transport in nature presumably (Orten 1966).

The level of zinc in blood and blood elements varies with age. Henzel et al. (1967) suggests that the erythrocyte zinc

content of newborn infants approximates one quarter the value for adults and rises to approach the latter level during the first twelve years of life. No detailed study of changes in normal zinc blood levels during adulthood has been conducted.

The fraction of ingested zinc which is not excreted and is not taken up by intravascular cellular components comes into equilibrium with plasma zinc, usually within 8 to 36 hours as a large pool of exchangeable zinc (Hœnzel et al. 1967). Studies with isotopic zinc have demonstrated that there is a continual tissue deposition and turn over of zinc which vary greatly with different tissues. Injected zinc 65 is deposited most rapidly and has the highest turn over rate in pancreas, liver, kidney, pituitary and adrenals. Muscles, testes and the Central Nervous System exchanged zinc less rapidly whereas zinc is deposited in the skeleton and teeth relatively slowly but is bound for a relatively long period of time. Zinc is also deposited in the hair and is retained until the hair is shed (Orten 1966).

Zinc has now been shown to be a constituent of a number of metalloenzymes. These include carbonic anhydrase (0.3 per cent zinc), pancreatic carboxypeptidase, liver and yeast alcohol dehydrogenase, glutamic dehydrogenase, lactic dehydrogenase, and probably other pyridine nucleotide - dependent metallodehydrogenases. In addition, zinc increased the activity of a number of other enzymes apparently as a "Co factor" in a non-specific manner. These include, arginase, emolase, yeast aldolase, axalacetic decarboxylase, lecithinase, histidine deaminase, carnosinase, amino peptidase, tripeptidase, dehydropeptidase, and glycl-glycine, glycyl L - leucine, alanylglycine and leucyl-glycine dipeptidases.

It is generally accepted that the entire body of a 70 kg

normal adult human contains approximately 2.2 grams of zinc, which is about one half the total body store of iron.

The mechanisms involved in zinc metabolism are not yet clearly understood. At best, the situation can be summarized as provided by Underwood (1971)

> "Injected Zn65 combines initially with the plasma proteins to form a plasma zinc pool and more slowly with the intracellular proteins of the cellular components. Clearance of plasma zinc, but not of erythrocyte zinc, is rapid to the soft tissues of the body and to the faeces via the small intestine."

V. METABOLIC FUNCTION OF ZINC

The study of metabolic function of zinc involves a biochemical study of the roles in which zinc may participate in metabolism by means of zinc - containing and zinc - activated enzymes, together with the information relating to the metabolic derangements in zinc - deficient animals or lesser biological systems. A critical review of these studies is beyond the scope of this thesis, and not necessary for its specific objectives.

According to Fox (1970), from the present stage of knowledge, it can be said that zinc functions by binding to molecules in the biological system to establish and maintain a balance necessary for chemical and physical function. Some of the studies have demonstrated relationships between zinc metabolism and structure of proteins, structure of nucleic acids, transport and release of metabolites or large protein molecules such as enzymes and the protein hormones; and synthetic and catabolic reactions in the body. Most of these relationships are not ubiquitously established. Further research is required to interpret these relationships in terms of bodily functioning.

VI. DIETARY FACTORS INFLUENCING AVAILABILITY AND UTILISATION OF ZINC

Even though a person were consuming the appropriate amount of zinc in his diet, all the amount of zinc that went into his body would not be available for absorption. There are some components of the diet which have a profound effect on the availability of dietary zinc. These factors, some of them well documented, and some yet to be confirmed, affect the availability of zinc by influencing the absorbability of the metal.

The first dietary component which came to the attention of researchers was phytic acid. Phytate can be found in plant protein, cereal grains and legumes. O'Dell and Savage (1960) show that phytate decreases zinc availability in their experiments into the role of zinc in the growth of chicks. They add phytate to a casein-gelatin semi-purified diet and demonstrate decreased growth among the chicks in a similar manner to those fed with a soy-bean protein diet, rich in phytic acid. This reduced growth symptom in both instances can be corrected by adequate zinc diet supplementation. Oberleas, Muhrer and O'Dell (1966) confirm this finding in the rat and conclude that phytic acid binds zinc into insoluble and non-absorbable zinc phytate complexes in the gastrointestinal tract which renders the zinc unavailable. They also look into the influence of calcium which, in excess, was earlier found to accentuate zinc deficiency syndrome in rats. Their results indicate that increased calcium intake accentuates decreased zinc availability in phytate containing diet by forming calcium-zinc phytate complexes which are less soluble than zinc phytate or calcium phytate alone. Similar results have been obtained in studies using a wide range of experimental and domestic animals. It is undoubtedly of significance in studies of zinc

deficiency in human subjects due to the extensive use of plant protein and grains in humans. However, no supporting data have yet appeared.

High calcium intake by itself has been shown to potentiate the zinc deficiency syndrome in pigs, dogs, and birds (Underwood 1971). The major but not the only site of calcium-zinc interaction is at intestinal level, resulting in reduced zinc absorption. However, such a calcium-zinc antagonism cannot be demonstrated in man. Spencer et al. (1965) conducted a study of radiozinc, Zn65 absorption in five normal subjects during periods of different calcium intake. The average intestinal absorption of Zn65 during period of low calcium intake was found to be 35.7 per cent. They did not find significant differences in absorption level during periods when calcium intake was increased six to ten fold.

The discovery of zinc deficiency syndrome in the Middle East to be described later, raises the interest in the effect of geophagia, the practice of clay eating, on the availability of dietary zinc. Geophagia is common in many parts of the world including the Middle East. Even though there was no evidence of it in Egyptian subjects, all Iranian subjects studied presented a history of geophagia. The degree to which clay makes zinc unavailable is not known. Clays from different locations have been shown to bind iron into unabsorbable complexes in varying degrees (Minnich et al. 1968). It is probable that similar effect exists with zinc. Fox (1970) postulates that, in the case of Middle Eastern villagers exhibiting zinc deficiency syndrome, whose diet consists mainly of plant protein in the form of whole wheat bread rich in phytic acid, the large amount of calcium in the clay may act in a similar way to calcium salts to prevent zinc

absorption in the manner described above, viz forming calcium-zinc phytate complexes.

There are evidences of mutual antagonism between zinc and copper in man and animals. Pfeiffer and Iliev (1972) demonstrate excessive levels of copper and low levels of zinc in schizophrenia. Women on oral contraceptives have been shown to have lowered plasma zinc levels (Halsted and Smith 1970) while having raised plasma copper levels. Cox and Harris (1959) produces copper deficiency anaemia among rats fed diets containing 0.6 per cent zinc for three weeks. Their finding is supported by Lee and Matrone (1969) who find that high zinc diets produce a precipitous drop in the copper transport protein, ceruloplasmin levels within one week. On the other hand, Van Campden (1969) showed that zinc absorption was reduced when the rats are given copper intraduodenally. In this elaborate experiment, Van Campden places Zn65 into isolated duodenal segments of the rat and administers copper either intraduodenally or intraperitoneally. Zinc absorption by the intestine is reduced with the former treatment but not the latter indicating that copper interference with zinc uptake is mediated at the intestinal level.

Two drugs are now known to affect zinc availability, D-penicillamine (β,β-dimethylcystine) used in treating Wilson's disease and cystinuria and ethambutol (2, 2' (ethylenediimino) - di-butyric acid) used in treating tuberculosis. D-penicillamine is normally administered to reduce the excessive amount of copper in the body in cases of Wilson's disease. It is found to produce abnormal excretion of zinc as well as copper (McCall et al. 1967). Similarly, Ethambutol has been shown to cause losses of copper and zinc in the dog, monkey and rat (Buyke, Sterling and Peets 1966).

Other dietary components said to affect availability of zinc are the non-essential, toxic element cadmium (Schroeder 1967) and EDTA. Cadmium is found to aggravate zinc deficiency in poultry, pigs and calves and appears to compete with zinc at important cellular binding sites (Underwood 1971). EDTA (ethylenediaminetetraacetic acid) has been found to increase the availability of zinc from soy bean protein diet among turkey poults (Kratzer and Starcher 1963). EDTA is the synthetic chelating agent commonly used in foods to decrease deterioration caused by heavy metals. The action of EDTA on availability of zinc is still not clearly understood. Darwish and Kratzer (1965) showed that EDTA could be absorbed but it was not possible to show that Zn-EDTA complex was absorbed.

Furthermore, the degree of zinc absorption appears to vary with the chemical form or combination in which it is ingested. Zinc in the form of zinc carbonate, zinc lactate, zinc sulphate, zinc oxide and zinc metal seems to be equally available to animals when included in their diet but zinc in naturally occurring ores, such as sphalerite and franklinite is largely unabsorbed (Edwards 1959).

VII. ZINC DEFICIENCY AND ALTERATIONS IN ZINC STATUS IN DISEASES

The investigations of zinc metabolism in clinical practice can be divided into two areas, those investigating the symptoms and treatment of zinc deficiency syndrome and secondly, those investigating changes in zinc status as a result of diseases and other unusual physiological states. Both types of studies involve the evaluation of zinc status in the human body at different stages of the disease. It is therefore, appropriate

to consider the criteria and techniques used in evaluating zinc status before detailing the findings of these investigations.

(1) Criteria for evaluating zinc status

Earlier study of zinc metabolism employs the balance techniques common in the studies of other nutrient requirements. The study of McCance and Widdowson (1942), highly regarded by other researchers in this area as pioneering, is a good example of this technique. They observed the output of zinc in faeces and urine during periods of different zinc intake levels. The balance between input and output, they suggested, was an indication of zinc status in the body. The clinical value of such a study is limited. This technique does not provide an evaluation of absorption. The balance for short periods is a physiological average that reflects immediate intake, immediate needs and available body stores. Furthermore, it is not practicable, if at all possible to keep an account of all intake and excretion of the metal by various means, in and out of the body.

However, balance studies have yielded useful information. McCance and Widdowson (1942), as a result of their above mentioned study, stated that the normal person excreted 0.3mg of zinc per day in the urine, although this amount did not change significantly with the intake by mouth, nor was it appreciably raised by intraveneous injections. They also stated that patients with albuminuria excreted seven times the normal amount of zinc in their urine. Other researchers have utilised this finding that urinary zinc excretion rate is stable and independent of intake fluctuations, to study zinc status in different diseases. Abnormal amount of urinary zinc excretion has been demonstrated in patients with Wilson's disease being treated with D-Penicillamine (McCall et al. 1966), post operative patients (Lindeman et al.1972),

severe burns (Nielsen and Jemec 1968) etc. However, as Lindeman et al.(1972)point out, urinary excretions vary widely among individuals and may be unreliable as an indication of zinc status.

As mentioned earlier, zinc is also contained in sweat. A small number of studies compare zinc excretion in sweat in different diseases. Prasad et al. (1963) found zinc concentration in sweat from dwarfs to be half that of control subjects. Collection of sweat presents technical problems and usually necessitates exposure of the subject to excessive temperatures, which raises problems in interpretation of the physiological significance of the values obtained.

Zinc - 65 turn over studies have been performed among a limited number of normal individuals, dwarfs and patients suffering from serious diseases. Prasad et al. (1963) found a more rapid uptake of zinc by the tissues of dwarfs than by those of normal subjects. But as Fox (1970) suggests, Zinc - 65 is a strong gamma-emitting radioisotope with a long half-life of 245 days. It's biological half-life in human beings was found to average 154 days and therefore, has limited suitabilities for studies with human beings.

Some investigators have found that zinc concentration in hair is a good indicator of zinc status. Orten (1966) states that zinc is deposited in the hair and is retained until the hair is shed. Clearly, the problem of absorbed zinc from external sources, such as sweat and hair dyes, must be considered in any study of hair zinc levels. Many studies in literature have used hair-zinc as indication of zinc deficiency. Reinhold et al. (1966) regarded a value of 127 ppm or less as indicating zinc deficiency. Strain et al. (1966) report reduced hair zinc among ten zinc-deficient Egyptian male dwarfs when compared with eight other

dwarfs treated with zinc. The reliability of matched groups comparison employed in these studies is questionable when one looks at the conflicting evidence in literature concerning hair zinc levels in normal humans and those with probable zinc deficiency. Some of this evidence is shown in Table 1. Zinc hair level regarded as normal by one investigator is lower than the level obtained from zinc deficient subjects by other investigators.

TABLE I. HAIR ZINC CONCENTRATION AS REPORTED BY VARIOUS INVESTIGATORS

Investigators	Subjects	Hair Zinc, ppm
Strain et al. (1966)	10 Zinc-Deficient Egyptian Male Dwarfs, 16 to 20 years	54 ± 6 [a]
	8 Zinc-Treated Dwarfs, 16 to 20 years	121 ± 5 [a]
	12 Normal Egyptians, 27 to 40 years	103 ± 4 [a]
	6 Normal Rochester Residents, 23 to 37 years	120 ± 5 [a]
Reinhold et al. (1966)	19 Zinc-Deficient Male adult Iranian villagers	139 ± 16.4 [b]
	20 Male control adult subjects	181 ± 36.3 [b]
Klevay (1970)	31 Panamanian males, 6 to 10 years	127 ± 49 [b]
Eminians et al. (1967)	Control village Iranian male children, mean age 10.8 years	163 ± 22 [a]
Trace Element Laboratory, Washington, D.C. *	14 Normal adults, Washington D.C.	176 ± 37 [b]
McBean et al. (1971)	75 Iranian children aged 6 to 12 years	199 ± 22 [b]

a Values are mean ± SE

b Values are mean ± SD

* Quoted from McBean et al. (1971)

The validity of hair-zinc concentration as an indication of zinc status is further put in doubt when studies exploring the connection between hair-zinc and zinc serum or plasma levels fail to produce significant correlation. McBean et al. (1971) found a correlation of 0.05 between hair and plasma zinc concentrations from the same individual among 75 Iranian children aged six to twelve years.

The most widely used index of zinc status is plasma and serum zinc levels. The content of zinc in the blood has been discussed earlier and it has been mentioned that there is a recent evidence suggesting that the zinc content of serum averaged 16 per cent higher than that of plasma from the same sample of human blood (Foley et al. 1968). This finding comes only from one study. To date, serum and plasma zinc contents are still commonly regarded as equivalent and the two terms are used interchageably by many investigators.

For a long time, the measurement of plasma and serum zinc has been regarded as unreliable. Henzel et al. (1967) quote figures for plasma zinc levels as ranging between 80 μg/100ml and 390 μg/100ml. Such a wide variation, they suggest, is the result of differences in the methods of analysis of the blood sample and the experience of the analytical chemist. With the recent introduction of the atomic absorption spectro-photometric technique of anlysis, which is now being used universally, it has become possible to measure zinc concentration in plasma and serum with greater accuracy (within 1 microgram per 100ml). A list of serum and plasma zinc levels in normal human subjects taken from more recent publications are shown in Table II.

TABLE II. PLASMA AND SERUM ZINC LEVELS OF NORMAL SUBJECTS FROM DIFFERENT SOURCES

Investigators	Subjects	Serum/ Plasma	Mean ± 1 S.D. (μg/100ml)
Kahn et al. (1965)	64 normal control age 22 to 73 years	serum	90.6 ± 16.7
Sullivan & Lankford (1968)	24 male staff physicians	serum	94.0 ± 11.0
Prasad (1967)	17 Iranian villagers	plasma	108.0 ± 8.0
Greaves & Boyde (1967)	41 normal control age 19 to 64 (mean 30.6) years	plasma	118.3 ± 12.5
MacMahon et al. (1968)	normal Australian adults	plasma	90.0 ± 18.0
	infants aged under seven months (Australia)	plasma	75.0 ± 9.8
Davies, Musa, Dormandy (1968)	67 healthy doctors and nurses, age 19 to 58 years male	plasma	96.0 ± 10.5
	female	plasma	95.0 ± 13.0
Halsted et al. (1968)	51 control subjects age 26 to 62 years	plasma	96.0 ± 13.0
Halsted & Smith (1970)	89 adults age 26 to 62 years	plasma	96.0 ± 12.0
	26 children age 3 to 13 years	plasma	89.0 ± 13.0
Sinha & Gabriel (1970)	200 control subjects	serum	120.0 ± 22.0
McBean et al. (1971)	75 Iranian children age 6 to 12 years	plasma	72.0 ± 12.0
Lindeman et al. (1972)	204 control subjects mean age 50 years	plasma	96.0 ± 1.2

These studies employ atomic absorption spectro-photometry as the method of analysis. It can be seen that the variation is not as wide as previously mentioned. The differences among figures from different sources can perhaps be attributed partly to factors affecting zinc levels in normal subjects.

An example of such factors has been provided by Davies et al. (1968). They determined plasma zinc values in normal men and women who had fasted overnight. The subjects were then given 50 grams of glucose in 100 millilitres of zinc free water. Plasma zinc levels fell within 10 minutes and reached minimum levels at one and a half hours, then rose to about the original levels by two hours. Well controlled diabetics that did not require insulin therapy had similar zinc curves. Intravenous injections of glucose (0.5 grams per kilograms body weight) had a similar but more rapid effect on plasma zinc levels. This result demonstrates the importance of dietary intake causing changes in metabolic rate in the determination of zinc plasma levels. This factor must be taken into account when one compares plasma zinc levels at different stages of a study, Similar effect of dietary intake on plasma zinc levels have been shown in calves, lambs and rats fed diet free of zinc (Fox 1970).

For plasma and serum zinc values to be meaningful, rigid precautions must be taken to avoid contamination of the sample by the syringe, the needle, the centrifuge tube and the storage container. This is because of the abundance presence of zinc in the environment. Also, as pointed out earlier, the red and white blood corpuscles contain appreciable amounts of zinc, therefore hemolysis and delay of centrifugation must be avoided.

Plasma and serum zinc values have been used extensively in the study of a wide array of human diseases, to be detailed

later. The interpretation of these levels in terms of overall zinc-status presents a problem. Most researchers agree with Pfeiffer and Iliev (1972) when they say

> "Plasma or serum zinc levels do not, in our experience, provide a valid index to the tissue zinc levels or the degree of deficiency which may exist".

Henkin (1974) while reviewing the role of zinc in wound healing, agrees with this, stating that in acute or chronic infectious disease processes, anaemias, drugs of various types, liver diseases, malignant and other processes, serum zinc levels may be depressed affecting a redistribution of body zinc pools, without necessarily producing a total body loss or a total state of deficiency. Clearly, a direct method of assessment of total body zinc status is needed, so far no such method is available. Many compromises have been suggested but none of them is satisfactory. Henkin (1974) suggests a measurement of zinc urinary excretion combined with zinc serum or plasma level as a more valid index. Here again, many pitfalls in interpretation occur, in many cases the two measurements are not related. For example, in case of decreased food intake or starvation or post-operative patients, urinary zinc excretion is greatly elevated while serum zinc remains stable. The combination of urinary zinc excretion and serum levels will lead to more confusion rather than clarity.

Hair zinc and plasma zinc levels combined do not give a clearer picture either. McBean et al. (1971) found a correlation of 0.05 between the two levels from the same child among the 75 Iranian children they studied.

Therefore, until a more accurate index of total body zinc is available, serum and plasma zinc levels still remain a good research tool in the study of zinc status. They are an indication

of the amount of available zinc in the body and also important indication of absorption in cases where zinc supplement therapy is being given. However, the limitation in interpreting these levels in terms of deficiency must be fully realised and any temptation to make generalisations regarding total body zinc status, avoided. A great deal of work still needs to be done to establish conditions that can alter serum and plasma zinc levels in normal persons before it can be used to characterise abnormal conditions.

(2) Human Zinc Deficiency

Zinc deficiency is known to produce retardation of growth and testicular atrophy in animals. Zinc deficiency in rats results in testicular atrophy with other symptoms in mouse, rat and pig including lack of growth and retardation of skeletal maturation. Changes in alkaline phosphatase, a zinc containing enzyme, have also been observed in pigs with zinc deficiency. Increasing activity of this enzyme has been noted when zinc deficient animals receive increased amounts of dietary zinc. This knowledge led to the diagnosis of zinc deficiency among Middle Eastern villagers in the early 1960's.

Prasad et al. in 1961 reported a syndrome affecting males in Iran characterised by severe iron-deficiency anaemia, hepatosplenomegaly, short stature, and marked hypogonadism. The diet of these patients consisted almost exclusively of bread made of wheat flour and the intake of animal protein was negligible. They all gave a history of geophagia. There was no evidence of blood loss or hookworm manifestations. Following therapy with pharmaceutical ferous sulphate (which contained appreciable quantities of zinc) and a good hospital diet, the anaemia was corrected, their hepatosplenomegaly improved, they grew pubic hair

and their genitalia size increased. Serum alkaline phosphatase was also found to increase following this treatment (Prasad, Halsted and Nadimi 1961). However, the doctors found it difficult to explain all the clinical features on the basis of tissue iron deficiency alone. On the basis of findings from animals with zinc deficiency mentioned above, the possibility that zinc deficiency may have been present was considered.

The diagnosis of zinc deficiency was used to describe the above syndrome when the same group of physicians encountered similar patients in Egyptian villages (Prasad et al. 1963). In Egyptian cases, the diet consisted mainly of bread and beans, the intake of animal protein was negligible. The clinical features were remarkably similar to the Iranian cases except that the Egyptian patients gave no history of geophagia but instead had schistosomiasis and hookworm manifestations. The Egyptian patients were demonstrated to have zinc deficiency in addition to iron deficiency anaemia on the basis of the following findings:

(a) the zinc concentrations in plasma, red blood cells and hair were decreased.

(b) the excretion of zinc in urine and sweat was decreased.

(c) the radioactive Zn65 studies which revealed that the plasma zinc turnover rate was greater in the patients, the twenty four hour exchangeable zinc pool was smaller and the excretion of Zn65 in stools and urine was less in the dwarfs than the control subjects (Prasad et al. 1963).

Under the experimental conditions, when the patients were divided into groups receiving treatments with zinc and/or iron, the rate of growth was greater in patients who received supplementary zinc as compared to those who received iron instead. Sexual maturation increased rapidly, normal pubic hair, genitalia

size and secondary sexual characteristics developed within twelve weeks after zinc supplementation was started. No such progress was obtained in the group treated with reagent grade iron medication. The result of zinc supplement therapy can be interpreted as confirming the diagnosis of zinc therapy in these patients.

Coble et al. (1966) presented evidence against the above findings. They reported a follow up study of patients with dwarfism and hypogonadism from an Egyptian oasis previously studied by Prasad et al. They found that among the majority of the dwarfs, increase in growth and gonadal development could be demonstrated within 3 years without any treatment and during this time plasma zinc concentrations which were low were not altered. They concluded that these cases merely represent examples of delayed maturation and not zinc deficiency. The stability of plasma zinc levels when growth was increasing, to them, indicated that zinc did not play a part in the development.

Prasad (1967) dismissed this claim saying that plasma zinc concentration alone was not a valid index of zinc deficiency state, but if one chose to accept this index as being valid, he said, one could interpret Coble et al's findings as showing a correlation between low plasma zinc and slower growth and delayed sexual maturation.

More recent evidence is in Prasad's favour. Halsted et al. (1972) studied fifteen Iranian male dwarfs aged 19 and 20 years old who were rejected by the Iranian Army Induction Centre because of "malnutrition". They also studied two women, a unique feature as previous studies were only conducted on male subjects. These patients, both male and female, exhibited signs of zinc deficiency syndrome, described above. They were studied for six to twelve months, being divided into three experimental groups. One group

was given a well balanced nutritious diet containing ample animal protein plus a placebo capsule. The second group was given the same diet plus a capsule of zinc sulphate containing 27 milligrams of elemental zinc daily. The third group was given the diet alone without additional medication for six months, followed by the diet plus the administration of zinc sulphate. They found that though the subjects receiving the diet showed improvement, the effect on height increment and onset of sexual function was significantly enhanced in those receiving zinc medication. This result confirms the role of zinc in growth and maturation in human subjects.

Similar studies with similar results have been conducted in Turkey, Morocco, Tunisia, Portugal and Panama. From these reports, the etiological factors in nutritional dwarfism associated with zinc deficiency seem to comprise the following:

(a) Unavailability of zinc; the subjects of these studies have inadequate diet consisting mainly of plant protein; bread made with wheat flour in Iran and bread and beans in Egypt. The intake of animal protein is minimal. The ingestion of zinc is reduced and is further complicated by the action of phytate in plant protein as mentioned earlier.

(b) Loss of zinc; many of these patients have chronic blood loss through parasitic manifestations. This presumably, reduces the amount of zinc available in the body. These patients come from hot climates where loss of zinc through sweat is considerable.

(c) Other factors such as geophagia, and liver function abnormality are common in these patients. These may play a significant role in zinc deficiency.

(3) Changes in Zinc Status in Pathologic Conditions

A great deal of research has been done on the alterations of zinc status due to several pathologic conditions. From time to time, attempts have been made to determine zinc levels with the purpose of employing these levels as diagnostic tools in different conditions, but most of these studies were limited in scope, often resulting in conflicting reports. The role of zinc as an essential component of several hepatic enzymes, including glutamic and alcohol dehydrogenases prompted several workers to investigate the metabolism of zinc in alcoholism and in other hepatic diseases. Successes of zinc therapy in wound healing led to the investigations of zinc metabolism in related conditions including surgical patients and patients with thermal burns. Assorted other pathologic conditions have been found to affect zinc metabolism such as anaemia, hyperthyroidism, hypertension and atherosclerosis. A brief review of zinc metabolism changes in relevant conditions will be discussed here.

(a) Zinc Metabolism and Hepatic Dysfunctions. Vikbladh (1951) determined serum zinc in various diseases of the liver and found that serum zinc is low in acute hepatitis, cirrhosis, and obstructive jaundice. The number of cases investigated were small, and as such, no definitive conclusions were drawn. The presence in the liver of alcohol dehydrogenase and glutamic dehydrogenase, both zinc metalloenzymes essential for the metabolism of alcohol in the body led Vallee and his co-workers (1956, 1957, 1959) to investigate the metabolism of zinc in patients with alcoholic cirrhosis. They reported a decreased concentration of serum zinc which correlates positively with the severity of hepatic dysfunction. At autopsy, they found significantly decreased quantities of zinc in liver tissues in

five patients. They also noted that the mean excretion of urinary zinc of ten patients with severe alcoholic cirrhosis was greater than that of a control group.

Vallee et al. (1957) demonstrated that the alcohol dehydrogenase of horse liver shows increased activity in the presence of ethyl alcohol to a concentration of 0.8 to 0.9 micrograms per millilitre. Concentrations greater than this cause inhibition of the enzyme activity. Approximately a fifty per cent decrease from the maximal activity is noted at a concentration of 4.6 micrograms per millilitre of alcohol, a blood level consistent with severe intoxication. This result has enabled Vallee to form a hypothesis explaining the participation of alcohol in the development of chronic liver disease. In essense, this hypothesis considers the possibility that continued high blood alcohol concentration may lead to inactivation of the alcohol dehydrogenase, increased urinary excretion of zinc and low serum zinc levels with disruption of other zinc-dependent enzyme systems, including those important in protein metabolism such as glutamic dehydrogenase. Post alcoholic cirrhosis, resulting from continual exposure of liver tissues to unconverted alcohol, may be considered a conditional zinc deficiency in the light of this hypothesis.

Sullivan and Lankford (1962) subsequently reported increased zinc excretion in patients with alcoholic cirrhosis as well as in chronic alcoholics without evidence of abnormal liver function and presumably, without cirrhosis of the liver.

Sullivan and Lankford further extended their investigation of zinc metabolism in chronic alcoholics who do not show any sign of abnormal liver function in 1965. They studied 124 patients admitted into a hospital with acute or chronic alcoholism and

concluded that in chronic alcoholics, the incidence of decreased serum zinc concentration, excess urinary zinc excretion and increased renal clearance of zinc is high. In the majority of the patients, the abnormal zincuria is transient, with zinc excretion returning to normal in one or two weeks following abstention from alcohol and an adequate diet. The alteration in zinc metabolism is apparently restored readily in most alcoholic subjects in contra distinction to those with post-alcoholic cirrhosis, in whom marked zincuria may persist for long periods (Sullivan 1962). They however, pointed out that neither the duration of chronic alcoholic ingestion or the initial level of blood alcohol could be correlated with abnormal zinc excretion.

Patients with other types of liver diseases including postnecrotic cirrhosis, viral and drug induced hepatitis, metastatic carcinoma have been found to have decreased zinc serum or plasma levels (Halsted et al. 1968, Halsted and Smith 1970). Kahn et al. (1965) reported that in patients with various types of liver diseases, low serum zinc as well as elevated urine zinc was seen most often in patients who were clinically more ill. They divided their subjects into "compensated" and"decompensated" cirrhosis groups. The "compensated" patients had neither significant jaundice nor ascites, while "decompensated" patients had either or both of jaundice and ascites.

Attempts at using zinc levels for diagnostic purposes have not been successful. Helwig et al. (1966) investigated the probability that measurement of urinary and serum zinc levels might provide a presumptive test for persons with cirrhosis of the liver, including cirrhosis associated with alcoholism, and with chronic alcoholism in the absence of overt cirrhosis. They did not find significant differences in serum and urinary zinc levels

among different categories of alcoholic patients (patients with definite liver malfunction, suspected liver malfunction, possible liver malfunction, and no liver malfunction), and also when comparing the alcoholics' zinc levels with those of normal subjects. This result is in contrast to other published results. Their patients were in better physical condition than those studied by other investigators and the results could perhaps be interpreted in the light of Kahn et al. (1965) findings.

From the available data, abnormality of zinc metabolism is seen to be associated with liver diseases. The mechanism concerned is not yet fully understood but is assumed to involve the activities of the two zinc containing hepatic enzymes, alcohol dehydrogenase and glutamic dehydrogenase. In the case of chronic alcoholism, continual ethanol assaults and the associated malnutrition are seen as factors affecting the amount or structure of these enzymes resulting in lower blood zinc levels and greater urinary zinc excretion. Prolonged malfunctioning of the enzyme alcohol dehydrogenase led to incomplete ethanol metabolism allowing the alcohol to damage the liver tissues resulting in alcoholic (Laennec's) cirrhosis. A table summarising the results of investigations on zinc levels in hepatic diseases is provided below.

TABLE III. SERUM OR PLASMA ZINC LEVELS IN HEPATIC DISEASES

* denotes plasma zinc values otherwise values shown are serum zinc levels

Investigators	Subjects	Mean Levels (µg/100ml)
Vallee et al. (1956)	Controls (age 20-51 years)	120.0 ± 19.0
	Severe Laennec's Cirrhosis	66.0 ± 19.0
	Mild Laennec's Cirrhosis	87.8 ± 20.0
	Infectious Hepatitis	107.0 ± 25.4
Sullivan and Lankford (1965)	Male Housestaff Physicians	94.0 ± 11.0
	Alcoholic Patients	64.0
Kahn et al. (1965)	Controls (age 22-73 years)	90.6 ± 16.7
	Compensated Alcoholic Cirrhosis	93.5 ± 22.4
	Decompensated Alcoholic Cirr.	78.9 ± 12.7
Helwig et al. (1966)	Controls (age 22-73 years)	91.0 ± 16.7
	Group I Alcoholics	91.0 ± 21.5
	Alcoholics with:	
	Definite Liver Malfunction	80.0
	Suspected Liver Malfunction	103.0
	Possible Liver Malfunction	89.0
	No Liver Malfunction	80.0
	Group II Alcoholics	91.0 ± 36.0
Halsted et al. (1968)	Controls (age 26-62 years)	96.0 ± 13.0 *
	Alcoholic Cirrhosis	64.0 ± 13.0 *
	Other types of Liver Diseases (not associated with alcohol)	70.0 ± 13.0 *
Sinha and Gabrieli (1970)	Controls	120.0 ± 22.0
	Acute and Chronic Alcoholics	110.0 ± 26.0
	Laennec's Cirrhosis	103.0 ± 29.0
Halsted and Smith (1970)	Controls	96.0 ± 12.0 *
	Alcoholic Cirrhosis	63.0 ± 14.0 *
	Other types of Liver Diseases	70.0 ± 17.0 *
Boyett and Sullivan (1970)	Controls	75.5 ± 12.7
	Laennec's Cirrhosis	44.1 ± 20.0

(b) Zinc in Wound Healing. A great deal of interest in Zinc metabolism has been awakened by the claims of success in wound healing with the administration of zinc supplementation. Even though zinc has been involved in wound healing since ancient times, in the form of zinc oxide in various salves, ointments and lotions, the interest in oral administration of Zinc for the treatment of wounds has only been expressed relatively recently. Common to many discoveries in medical history, the discovery of beneficial effects of oral zinc in the healing of wounds was accidental. During studies of the effects of dietary amino acids on wound healing in rats, commercial beta-phenyllactic acid, an analog of phenylalanine was surprisingly found to increase the rate of healing over that in controls. Purified beta-phenyllactic acid had no effect and a zinc contaminant in the commercial product was eventually found to be the stimulant. This finding sets off a series of experiments on wound healing in rats and other experimental animals and eventually in man.

Success from experimental administration of oral zinc supplement in wound healing among animals led to its application in clinical practice. Pories (1967) reported accelerated healing of pilonidal sinus marsupialization wounds in healthy young airmen when they were given supplemental zinc in the form of zinc sulphate 220 micrograms orally three times daily. Accelerated healing was noted particularly during the stage of epithelization, fifteen or more days after operation. This result, at first was viewed with scepticism due to lack of controls in the experimental design. However, Henzel et al. (1967) confirmed this result in a controlled study of twenty young healthy airmen undergoing excision of pilonidal sinus tracts. It was found that healing rates were approximately one third slower for the untreated patients

than they were for the patients treated with zinc sulphate supplementation.

The exact role of zinc in wound healing has been investigated by many researchers. Most of these studies involve the use of radioactive zinc 65 or biopsies of tissues surrounding the wounds at different stages of the treatment. They commonly find an accumulation of zinc in the healing wound but as yet the function of zinc in wound healing is not clearly understood. It is assumed that zinc affects the wound healing rate in the same way as it influences cellular division through the synthesis of protein and its functions in connection to DNA and RNA metabolisms.

Oral zinc sulphate has been reported to accelerate healing of leg ulcers (Husain, 1969). In chronic leg ulcers, it has been shown that the patients have significantly lower plasma or serum zinc levels than those of control subjects (Halsted and Smith 1970). The zinc treated patients' serum zinc levels rose during therapy suggesting that a state of zinc deficiency may have existed before therapy was started but no correlation between the healing rate and the serum zinc levels can be established (Myers and Cherry 1970).

Not all treatment with oral zinc in wound healing however, has been successful. Barcia (1970) repeated Pories study of zinc sulphate treatment of young airmen with healing pilonidal sinus wounds and found no acceleration of wound healing.

This contrasting result led to the speculation of a hypothesis that zinc supplement therapy is only successful in wound healing when the patient has been zinc deficient prior to the treatment and no acceleration of healing rate will be seen if the patient has adequate zinc before the commencement of therapy.

Hallböök and Lanner (1972) found evidence in support of

this hypothesis. In their double blind study, the effects of zinc and placebo on the rate of healing of venous leg ulcers were studied. Analysis of their overall results, if all data are pooled, suggested that there was no significant difference between zinc and placebo in accelerating the rate of wound healing. However, when patients with lower concentration of serum zinc (serum zinc equal to or lower than 110 micrograms/100ml), were evaluated as a group, they demonstrated a statistically significant acceleration of the rate of healing, whereas no differences could be demonstrated in patients with what these investigators considered normal concentrations of serum zinc (higher than 110 micrograms/100ml). Such results suggest that zinc has a role in wound healing in zinc deficient states, but not in states of adequate zinc status.

Such a conclusion may be ideal as a guide line in the use of zinc supplementation in the treatment of wounds and related conditions e.g. burns, ulcers, and skin lesions. In practical terms this creates a difficult problem. How can one identify a zinc deficient state? The validity of serum or plasm zinc levels as indices of zinc status is questionable as discussed earlier. To be certain of zinc status one needs to conduct a full scale analysis of zinc availability and supply, the like of those done in the studies of Middle Eastern dwarfs by Prasad and his co-workers mentioned earlier. Secondly, if we are to accept serum or plasma zinc level as a rough indication of zinc status, what level can be used as norm? Hallböök and Lanner (1972) suggest serum level of 110 micrograms/100ml as being adequate status, but this is arbitrary. Researchers in the field of Zinc metabolism have not been able to agree on a figure which can be regarded as normal serum or plasma zinc level.

Many workers have suggested the use of normal control

subjects' levels as norm for each experiment. Others question this saying that it is not certain that the normal control subjects, though not exhibiting known signs of zinc deficiency, in fact have adequate zinc status.

While these questions are yet to be answered, abnormal levels of zinc have been found in patients suffering from many other pathologic conditions. The finding of high concentrations of zinc in certain tissues led to the interest in the role of zinc in cancer. (Cristol 1927, Labbe and Nepveux 1927). A significant decrease in serum zinc has been observed in myocardiac infarction (Wacker, Ulmer and Vallee 1956), protein deficiency, acute and chronic beriberi, pellagra and tuberculosis, to name a few.

When studying the reports of abnormal zinc levels in various pathological states, one must realise the problems of interpretation in reference to normality. Until a norm can be agreed to, what is classified as abnormal zinc level by one investigator may be regarded as normal by many others (Vallee 1959). So far as abnormally low zinc levels are concerned, Halsted and Smith (1970) perhaps have a practical approach when they say

> "An abnormally low plasma-zinc concentration merely suggests zinc deficiency, only a clear cut clinical response to zinc therapy under controlled conditions would constitute definitive evidence."

(4) Zinc in Psychology and Psychiatry.

The interest in Zinc in the field of Psychology is recent and as yet limited. Evidence of profound influence of zinc on behaviour potential has been obtained by Caldwall and Oberleas (1969). They compared the behavioural patterns of the surviving offspring of mildly zinc deficient mothers with similar young rats from Zinc supplemented mothers. The latter were found

to be significantly superior both in Lashley III water maze test and in a platform avoidance conditioning test. Oberleas et al. (1972) confirmed this result in their postnatal studies where observations were made on moderately severely deficient rats which were placed on the diet at 30 days of age, for forty eight days prior to testing these rats' performance were compared with paired-fed rats who were matched for weight prior to the start of dietary treatment. In all three measures of behaviour employed, namely open field, a platform avoidance conditioning test and Lashley III water maze, the performance of zinc deficient animals was significantly inferior to that of zinc supplemented animals. This superior learning ability, accompanied by larger "activity score" was interpreted as a reduced emotionality. It was emphasized that these effects of zinc deficiency upon behaviour were obtained on soy bean protein diets apparently adequate in protein.

In man, zinc has been found to play an important role in the normal functioning of gustation. This finding will be discussed in detail in another chapter.

In psychiatry, besides the studies of zinc metabolism in alcoholics, interest in the role of zinc in several psychiatric disorders have been expressed recently. The incidence of low zinc levels combined with excessive copper levels in schizophrenias has been investigated (Pfeiffer and Iliev 1972). These investigators found only 11 per cent of 240 schizophrenic outpatients to have low serum zinc levels (less than 80 micrograms/100ml). Their norm of serum zinc at 80 micrograms per 100 millilitres is however lower than that which is used by other investigators. The precise role, if any, of zinc in connection to schizophrenias is not known.

Some investigations are being carried out exploring the role of zinc in the hippocampal region of the brain where the highest brain level of zinc occurs. McLardy (1975) reported an abnormal granule-cell layer of the hippocampus in four out of eighteen brains from early-onset schizophrenics, and in all thirty brains from chronic alcoholics were used in his study. These abnormal brains were shown to have over 50 per cent subnormality in zinc content as compared with controls. These results are limited and as such no interpretation in behavioural terms can be made. However, further investigators in this area may yield more useful information into the role of zinc in some psychiatric disorders.

VIII. SUMMARY

In view of the present knowledge in Zinc metabolism discussed above, the following conclusion can be drawn.

(1) Zinc is widely distributed in foods, drinking water and the air.

(2) A well balanced human diet supplies some 10 to 15mg of zinc daily. The daily requirement of zinc in man is approximately 1 to 2mg.

(3) Absorption of zinc occurs in the intestine. Excretion is largely through the faeces with varying amounts through urine and sweat.

(4) Seventy five per cent of zinc in the blood exists in erythrocytes, 12 to 22 per cent in plasmaand 3 per cent in leukocytes. Varying amounts of zinc are deposited in bodily tissues at different turn over rates.

(5) The main metabolic role of zinc is seen as its functions through metalloenzymes in the structure of proteins and nucleic acids.

(6) Factors affecting the availability of dietary zinc includes plant protein containing phytic acid, other antagonizing elements such as copper and cadmium, geophagia and drugs.

(7) In view of the inadequacies of other indices of zinc status, zinc plasma or serum levels are seen as the most useful research tool in the studies of zinc metabolism. However, plasma and serum levels do not reflect tissue zinc status and as such interpretations of zinc plasma and serum levels in terms of total body zinc status must be limited.

(8) Zinc deficiency has been shown to result in nutritional dwarfism with severely retarded sexual development in

many countries. This syndrome can be corrected to a large extent by zinc supplementation.

(9) Abnormality of zinc status has been shown in various pathologic conditions including various types of liver diseases, alcoholic patients with and without liver malfunctioning and patients with chronic leg ulcers.

(10) The role of zinc in psychology and psychiatry, similar to many aspects of zinc metabolism, is not yet clearly understood and a great deal more work needs to be done to answer the many problems which still exist in this area.

CHAPTER III

TASTE, NORMAL AND ABNORMAL

Taste or gustation has often been described as a "poor relation" when compared with other human senses. This is because, when compared with the senses of vision and hearing, the volume of literature on taste is negligible. What one can gather from the available literature is an impression that taste, perceptually, is a complex sense. In addition to sensations arising from physio-chemical reactions in the taste receptors, taste perception is influenced by the action of other chemical, tactile, warm and cold receptors in the mouth, and in particular by olfactory sensations (Houssay 1955). Many of the controversies in the field of taste remain unresolved to date.

One of the most controversial issues is the problem of the number and identity of the primary taste modalities. Against much opposition, it is still widely held that there are but four primary taste modalities, sweet, salt, sour and bitter (Houssay 1955 Best and Taylor 1955, Fulton 1955, Bell, Davidson and Scarborough 1959 etc.). This view simplifies the complexity of the taste sense and makes objective studies more practicable. However, this simple model of four basic taste modalities has been criticized as inadequate and misleading. Evidence has been produced to oppose this view. Firstly, there are problems of nomenclature, where the use of four labels, sweet, salty, sour and bitter and even their combinations, has been proven to be inadequate in describing gustatory perceptual responses to some chemicals used in more recent studies.

Secondly, the formerly held belief that these four basic

taste qualities exist and that there are specific neural apparatus transmitting them, has been disputed. Pfaffmann (1941) found that when the neural signals passing along single units of the mammalian chorda tympani nerve (the nerve inervating the anterior region of the tongue, shown to transmit sensory impulses in gustation) were monitored, electrically, they were not specific to any one of the four classical taste qualities of sour, salty, bitter and sweet. This result implies that even when one looks at the four tastes, sweet, salt, sour and bitter as basic taste modalities, they still cannot be considered primary modalities since they are not totally independent of each other, in apparent contrast with the case of primary colours in vision. Also, this result shows that one taste neuron, or unit, may respond to several stimuli thought to represent different taste qualities.

Since that time, many other studies have been conducted employing sophisticated physiological techniques and models of data. The amassed data do not support the specificity view. In its place, the results seem to suggest a neural patterning view (Erickson 1968) which held that the higher brain centres decoded a neural pattern which contained the information about taste quality. Most taste nerve fibres respond to many types of prototypical stimuli but to varying degrees.

With these changes in a basic view of what constitutes salient features, the field of gustation has received increased attention in recent years. A number of excellent reviews on different aspects of the field have appeared (Amerine, Pangborn and Roessler 1965, Meiselman 1972). It is the purpose of the present discussion to present a selective account covering only a limited area of factors affecting taste perception both among normal individuals, for example the effect of age, sex, temperature

of tastants and among subjects who suffer from different ailments affecting their taste perception, for example, in the case of diabetes, pseudohypoparathyroidism, lingual lesions or metabolic and genetic disorders.

I. TASTE AMONG NORMAL SUBJECTS

The oldest and most common procedure employed in the study of the psychophysics of taste is the measurement of absolute thresholds. Two thresholds are normally involved; the "detection" threshold is the minimum detectable concentration at which the subject can reliably judge as "different from pure water", the other threshold, the "recognition threshold" is the concentration at which the specific taste can first be recognised. The inadequacy of this approach is that it equates detection sensitivity with performance in an overall sense, and this can be misleadingly simplistic.

Among normal subjects, i.e. subjects without any identifiable disease affecting their taste perception, studies have been conducted to determine threshold levels in response to a series of chemicals believed to represent each of the four basic tastes; for example, hydrochloric or citric acid for sour, sucrose for sweet, quinine sulphate for bitter and sodium chloride for salt. Thousands of taste thresholds are reported in literature. The data is not always comparable because of the differences in techniques employed, impurities in the chemicals, inadequate numbers of tests or insufficient statistical analysis of their validity, and the effect of undetermined factors such as order of presentation, temperature, extraneous noise, time of day, experience, physical condition, age, sex and area stimulated (Amerine, Pangborn and Roessler 1965). Even from day to day,

using the same subject and method, there are variations in the taste thresholds for a given compound.

Let us now look at some of the factors which affect taste thresholds in normal subjects.

(1) The Effect of Stimulus Delivery

Meiselman (1972) listed a selection of methods used in delivering the taste stimulus liquid to the tongue. These include

(a) brushing of stimulus onto the tongue where the stimulus is mechanically applied to the tongue. This procedure can be gross, involving a large tongue area, or can be as refined as delivery of one drop to one papilla.

(b) sipping, where the subject brings a glass or other container to his lips and either sucks in the liquid or pours the liquid over his tongue. The subject then either swallows the liquid or spits it out after tasting it.

(c) restricted dorsal flow procedures, where the delivery of a flow of stimulus to a portion of the tongue, usually a section of the anterior dorsal surface is involved. This can be accomplished with a pipette or medicine dropper, or with the use of a tongue chamber which constrains the flowing solution onto a smaller tongue area.

(d) anterior dorsal flow, where the subject extends his tongue and closes his lips on it, ensuring no saliva solution flows over the outstretched tongue.

(e) whole mouth flow, where a device, the Cornell Gustometer is used employing a dam constructed from dental impression compound, in which are embedded inflow and outflow tubes to deliver a constant flow of liquid to the entire mouth cavity and then remove the material by siphoning.

Each of these methods has its advantages and disadvantages, mostly concerned with the regulation of the flow of solution onto the tongue and the control of saliva. Research has not yet elucidated the characteristics of all procedures; the "sipping" and"drop" methods are more popular than the rest and some research results have indicated some differences in thresholds due to the differences between these two methods used to measure the thresholds.

Richter and Maclean (1939) illustrated the effects of the method of measurement in his experiment with sodium chloride. He employed four different methods. In the "drop" method, three drops were placed on the protruded tongue; in the "swallow" procedure, 10 millilitres were swallowed; in "comparison No.1", 10 millilitres of the salt solution were compared with 10 millilitres of distilled water; and in "comparison No.2", as much salt solution and distilled water as desired were available. The result showed that even though the "sensitivity" (detection) threshold was much lower than the "recognition" threshold for all methods, it was particularly so when unlimited quantities of salt solution and water were available for comparison. The "drop" method produced much higher thresholds than the "swallow" method in both sensitivity and recognition thresholds (sensitivity threshold averages:- drop 0.135%, swallow 0.047% ; recognition threshold averages:- drop 0.192%, swallow 0.167%).

This difference in thresholds between those obtained by the drop and the swallow or sipping method is to be expected when one considers the evidence provided by Smith (1971). He experimented with twenty four undergraduates at the University of Pittsburgh, determining taste intensity responses to several concentrations of sodium chloride, citric acid, quinine hydrochloride, and saccharin

for four differently sized areas of stimulation. Smith concluded that taste intensity is a power function of both area and concentration with the equation

$$I = C^n \times bA^p$$

describing the relation between intensity (I), concentration (C) and area (A). He further suggested that the differential effect between compounds upon taste threshold, due to the differences in the size of stimulated area, is not due to the various compounds as suggested by Bujas and Ostojcic (1941) and Hara (1955). Rather they said this differential effect on threshold is not a function of a differential effect of area but appears to be due to the unequal binding strengths for the various taste stimuli.

(2) The Effect of Sleep and Hunger

The effect of sleep and hunger on taste thresholds is complicated by other factors associated with them including the changes in bodily metabolism as a result of the lack of sleep and ingestion of food. Without concerning themselves with biochemical considerations, Furchtgott and Willingham (1956) found that lack of sleep up to 72 hours did not affect the thresholds to salt and sweet while lack of sleep for 48 and 72 hours raised the sour threshold significantly.

Yensen (1959), in his studies on hunger, found the sensitivity to the four basic taste qualities to be greatest at 11.30 a.m. There was a significant decrease in sensitivity for about an hour after a meal, followed by an increase in three or four hours. The degree of decrease in sensitivity appeared to be related to the caloric value of the meal and the type of meal ingested. Depletion of body salt content increased the sensitivity to salt but did not affect the other taste thresholds. Loss of body water caused a decrease in sensitivity to salt but

did not affect the sour threshold. Pangborn (1959) on a much larger group of subjects (over 1000) failed to replicate Yensen's diurnal effects. The effect of fasting on taste thresholds is still not clearly established. Meyer (1952) found no change in sensitivity to taste in up to 34 hours of fasting while Gusev (1940) seemed to find an increase in sensitivity one and a half and eight hours after fasting began. Furchtgott and Friedman (1960) reported that withholding lunch resulted in slightly lowered thresholds for sucrose, hydrochloric acid and sodium chloride.

Until such time as better methodologies are developed enabling complete control of the diet and allowing detailed biochemical, metabolic considerations to be taken into account, the effect of food ingestion, food deprivation and hunger on gustatory sensitivity will not be clearly established.

(3) The Effect of Age and Sex

The literature is not clear on what effect age and sex have on taste sensitivity. Beidler (1961 b) noted conflicting results on the effect of age on taste thresholds. The newborn seems to have little taste differentiation until about thirty five to forty days. However, Jensen (1932) demonstrated response to saltiness in children as young as two days old. Richter and Campbell (1940) found a much higher sweet threshold in a 52 - 85 year age group than in a 15 - 19 year age group. Cooper et al. (1959) supported this finding among their subjects whose ages ranged between 15 to 87 years. They found that curves for development and decline of sensitivity for the four basic tastes took the same form. Decline started in the late 50's and affected sour less than the other tastes. They produced a table of threshold values for the basic taste qualities in five age

groups which has been quoted widely as standard values. Degenerative changes in the taste receptors are believed to be responsible for this decline in sensitivity. According to Moore (1962), the number of taste buds per papillae drops from an average of 245 in young adults to 88 in subjects 70 - 85 years old.

Aubek (1959), while reporting significant decreases in sensitivity among subjects over the age of sixty observed no sex differences. In contrast, Tilgner and Barylko-Pikielna (1959) found woman to have a higher sensitivity than man for sweet and salty but less for sour and no difference between the sexes for bitter taste.

Glanville, Kaplan and Fischer (1964) found an interaction effect between age and sex. They reported that females were more sensitive tasters of hydrochloric acid at all ages. Maximum sensitivity to this compound was reached within the 16-20 year age group in males, followed by a decline of 0.071 threshold per year. In contrast, females showed little variation in sensitivity to hydrochloric acid with age. From the age of 16, sensitivity declined at the rate of only 0.018 threshold per year.

(4) The Effect of Smoking

Smoking is popularly believed to decrease taste perception. Bronte-Stewart (1956) hypothesized that smoking could affect taste preferences via the taste mechanisms. There are, however, few objective studies on the effect of smoking on taste. So far, the most authoritative study on the subject appears to be that conducted by Krut, Perrin and Bronte-Stewart in 1961. They tested 79 smokers and 77 non-smokers on a series of solutions of sucrose, citric acid, sodium chloride and quinine hydrochloride in tap water. They concluded that the taste thresholds for bitter

in smokers are higher than non-smokers. There was no significant difference in the taste threshold for sweet, sour or salt between smokers and non-smokers. They also found that the age of the smoker which they assumed indicative of the duration of smoking, as well as the amount smoked, both adversely affected sensitivity to quinine solutions.

The immediate effect of smoking, a great concern in taste experimentation, did not seem to influence taste thresholds in this work by Krut et al. (1961).

They said

> "The smoking of a cigarette has no immediate effect on taste, for any of the taste primaries in smokers. Smoking, in decreasing sensitivity to bitter appears to be the result of prolonged addiction to the habit".

(5) The Effect of Temperature

The effect of temperature on taste is of particular interest to the food industry. It is generally held that meats and vegetables have more flavour when hot than cold. As early as 1847, Weber reported that sensitivity of taste sensation was greatest when the stimulus was at the temperature of the body.

Goudriaan (1930) noted that an increase in temperature increased the sensation of sweet and sour, but decreased those of salt and bitter. In the case of mixtures, Moncrieff (1961) reported that sweet prevailed over sour but salt and bitter prevailed over sweet when the temperature is raised.

Amerine, Pangborn and Roessler (1965) while reviewing the literature on this topic said that optimum temperatures for taste sensitivity have been reported to be 35° - 50°C for sucrose and hydrochloric acid, 18° - 35°C for saltiness and 10°C for quinine.

The most recent study on the effect of solution temperature on taste intensities in humans is that conducted by Moskowitz (1973)

who concluded that alterations of stimulus temperatures leave unchanged the rate of growth in taste intensity with concentration. Only glucose and sodium chloride which he used to represent sweet and salty showed temperature dependency while citric acid and quinine sulphate did not. Also, only sodium chloride showed a consistent trend for intensity changes with temperature.

(6) The Effect of Cross-enhancement between Stimuli with Different Taste Qualities

One aspect of the studies of gustatory adaptation which concerns researchers arises in the situations where the adapting and test stimuli are of different taste qualities. This involves the investigation of interactions between different taste qualities including the enhancement of one quality by prior exposure of the tongue to a stimulus having a different quality. This is an important factor which needs to be taken into account when designing a gustatory experiment.

Kiesow (1894, 1896) reported the existence of cross-enhancement among all pairs of the four basic taste qualities. Subsequent studies (Dallenbach and Dallenbach 1943, Mayer 1927, Meiselman 1968) have supported the existence of cross-enhancement, with only one exception by Hahn and Ulbrich (1948) who found no changes in thresholds after adapting to substances with qualities different from that of the test solution. Gregson (1964,1966) indicated that some reported interactions were due to faulty scaling procedures. McBurney and Bartoshuk (1973), in reviewing the literature in this area, suggested that the difference in findings is due to the unique procedure employed in Hahn and Ulbrich's experiment. In order to prevent recovery from adaptation during the measurement of threshold, they mixed the substance to be tested in the adapting solution instead of water. This procedure

prevented the occurance of the "water taste", a phenomenon explained recently.

It is now generally accepted that water becomes a taste stimulus when it is presented following adaptation to many sapid substances. Bartoshuk, McBurney and Pfaffmann (1964) found that water has a sour-bitter taste following adaptation to a weak sodium chloride solution. They used this finding to explain the tastes often attributed to distilled water as being the result of sodium chloride adaptation of the tongue in the saliva. Since then, it has been shown that all four basic tastes can be produced in water by suitable adaptation (Bartoshuk 1968, McBurney 1969, McBurney and Shick 1971). Also it has been shown that the water taste of a compound will add to the taste of a weak solution that follows it (Bogart 1969, McBurney 1969).

The water taste phenomenon suggests a reinterpretation of those studies that found cross-enhancement. It may have been that the enhancement was produced by the water in which the second stimulus was dissolved rather than the solute itself. Recently, McBurney and Bartoshuk (1973) studied this new interpretation employing an experimental design which allowed independent measurement of the water taste of the adapting solution and the enhancement of the normal taste of the second solution. They examined all possible pairs of solutions of water, sodium chloride, urea, citric acid, caffein and sucrose. They concluded from their results that adaptation to a stimulus of one quality affects the taste of other stimuli through the addition of a water taste to the usual taste of the second stimulus, rather than by enhancement of the response to the second stimulus per se. In other words, following adaptation to a first taste stimulus, the taste of the water solvent in the second stimulus adds to the normal taste of

the solute in that stimulus. No evidence was found for true interactions among different taste qualities. Gregson (personal communication) does not subscribe to the water taste theory.

(7) Other Factors

Besides the factors mentioned above, there are other factors which are believed to affect taste perception among normal subjects.

In all threshold determinations, practice is a factor. One probably learns to fix one's attention on the proper taste so that taste cues are recognised at lower concentrations with increased familiarity with the stimuli (Pangborn 1959).

The effects of different drugs are not as yet fully investigated. Drugs which affect metabolic rate, for example tranquilizers and stimulants, may affect taste thresholds in some way. Penicillin compounds do affect taste perception.

The effect of the menstrual cycle on taste sensitivity has been investigated (Glanville and Kaplan 1965). Among 23 subjects tested for taste sensitivity to quinine sulphate through one or more menstrual cycles, it was found that sixty-six per cent of the subjects showed some increase in sensitivity, the extent of which varied greatly. Other subjects showed no change and a few declined in sensitivity. No other investigations in this topic have been reported.

Other factors investigated, because of their commercial uses in the food industries, include the effects of taste medium and the viscosity of the taste stimuli.

II. TASTE ABNORMALITIES DUE TO DIFFERENT DISEASE STATES

While much interest has been paid to the peculiarities of

responses to phenylthiourea or phenylthiocarbamide and other related chemicals, little attention has been given, until recently, to the effect of disease on taste perception. The earliest documented observation seems to be that of Cameron (1947) who reported eight cases of patients suffering from Multiple Gliomata and Tic Douloureux. Among these patients, sweet taste was totally absent with either delayed or absent sensory functioning in bitter, salt and sour.

It is only relatively recently that taste perception among patients with different confirmed clinical complaints has been investigated in depth. Several patient groups have been studied; some of the results will be discussed here.

(1) Abnormalities in the Oral Region

Traditionally, taste in man has been considered a function of the tongue. Tongue maps were drawn in which taste sensitivity in man was predicated upon electrophysiological thresholds obtained through recordings at one or the other of the nerves inervating the tongues of various animals after the placement of sapid substances on the lingual surface. In this old and inaccurate view, the tip of the tongue was considered most sensitive to the salt taste, the sides most sensitive to sour, the middle area most sensitive to sweet and the posterior third most sensitive to the perception of bitter. This view did not take into consideration the importance of other organs in the oral region, namely the palate, pharynx and larynx and their roles in taste processes.

This gap in knowledge has been filled by the study of Henkin, Graziadei and Bradley (1969). After selective anaesthetization of the tongue and palate, they found that the palate contributed significantly to the taste of sour and bitter and not to sweet and salt. When they completely anaesthetized both

the tongue and palate, they reported that normal subjects neither detected as different from water, nor recognised as salty, sweet, bitter or sour, saturated solutions of sodium chloride, sucrose, urea or a 0.3N solution of hydrochloric acid respectively. When these subjects were requested to swallow the various solutions presented to them, thresholds were obtained in the pharyngial area which were not particularly different from those found when the individuals were tested with their tongues and palates intact. The tongue by itself was found to be most sensitive to salt and sweet while bitter and sour could also be appreciated in this area.

With the above findings, Henkin et al. (1969) looked at patients who had some abnormalities in the oral region. These include

(a) Lingual Lesions. Patients with various lingual abnormalities were studied, for instance Lichen Planus, severe Moniliasis, Tumors that have extensively infiltrated the lingual epithelium, and patients after bilateral section or injury of the facial nerves supplying the tongue. These patients generally exhibit elevated thresholds for sweet and salt but normal thresholds for the tastes of sour and bitter. In other words, the patients act as if their tongues were functionally anaesthetized due to the destruction of their lingual taste buds and associated nerves by these various pathological processes.

(b) Dentures. Henkin et al. (1969) found that there is little difference in taste sensitivity between patients with tightly fitting maxillary dentures in place and normal volunteers after palatal anaesthetization. These maxillary dentures fully cover the hard palate and extend beyond the junction of the hard and soft palates. In these patients, median detection and recognition thresholds for salt and sweet are normal, but thresholds

for sour and bitter are markedly elevated. When the dentures are removed, sour and bitter acuity improves and usually returns to normal levels.

Some patients who have worn maxillary dentures chronically have elevated thresholds for sour and bitter with their dentures removed and exhibited even more impairment with their dentures in place. This, at present, is assumed to be due to damage to palatal papillae and taste buds resulting from mechanical irritation to the structures on the palatal epithelium, especially as the appliance loosens and irritates over time.

(c) Gonadal Dysgenesis. Patients with chromatin-negative Gonadal Dysgenesis, who generally have high arched palates with some having submucous clefts of the dorsal hard palate, exhibit abnormalities of taste. Their thresholds for salt and sweet are essentially normal, but their thresholds for sour and bitter are abnormally elevated. In general Henkin et al. (1969) found that these patients showed multiple abnormalities of sensation and perception over the hard palate, appearing as if their palates were anesthetized. It was noted that patients with gross clefts of the dorsal hard palate without other abnormalities of growth and development exhibit normal taste acuity for each quality. This seems to indicate that the patients with taste abnormalities may have some neurosensory abnormalities that may account for their abnormality of sour and bitter taste.

(d) Pseudohypoparathyroidism. In addition to having low serum calcium concentrations and elevated serum phosphorus concentrations, patients with pseudohypoparathyroidism exhibited elevated thresholds for the sour and bitter tastes but relatively normal thresholds for the salt and sweet tastes (Henkin et al. 1969). These patients also have anatomical and physiological

abnormalities of the palate which were assumed to account for their abnormality of sour and bitter tastes. One type of these palatal abnormalities is high and arched, like in gonadal dysgenesis; the other, which is more common, is elongated and extremely flat with excessive calcification.

(e) Type I Familial Dysautonomia (Riley-Day Syndrome).

The most interesting group of patients with abnormalities in the oral region is Riley-Day Syndrome. These patients exhibit ageusia, complete absence of taste responsiveness, or severe hypogeusia, that is decreased taste sensitivity. Untreated, most of these patients are unable to detect or even to recognise differences between water and saturated solutions of sodium chloride, sucrose or urea (Henkin 1967, Henkin and Kopin 1964). By careful examinations and biopsies, the only neural structure found in the anterior two thirds of the tongue and associated areas were those of unmyelinated nerve fibres. There is a total absence of the taste buds or papillae usually present in these areas. The unmyelinated nerve fibres do not terminate on any specialized epithelial structure. They are also fewer in number than those found in people with normal taste acuity. Lastly, there is no increase in number of the unmyelinated nerve fibres over the anterior two thirds compared with the posterior one third of the tongue, although this is the case in normal individuals.

It was found (Henkin 1969) that after parenteral administration of methacholine, the abnormally elevated thresholds for sodium chloride were lowered in each of the patients so treated. Not only could they detect sodium chloride at normal concentrations, but they recognised relatively dilute solutions as well as the more concentrated ones as salty. Some improvements on taste acuity for sucrose was also reported in

some cases. As the effects of the drug wore off, the patients became quantitatively less able to detect or to recognise either the sweet or salt taste. Within thirty minutes after injection, taste discrimination had returned to its initial abnormal level.

This finding is of great importance. The role of taste buds, previously believed to act as taste receptors needs to be reconsidered. With this evidence, it seems that taste buds only act as "chemical sieves" holding the taste stimulus in place to be in contact with the unmyelinated free nerve endings usually terminated in the taste buds. The finding that methacholine administration brought taste acuity to within normal levels leads to a new era of the treatment of taste abnormalities which will be discussed later.

(2) Other Disease States.

Besides the above mentioned groups of patients whose taste abnormalities seem to be caused by the abnormalities in the oral region, there are other patient groups who showed taste abnormalities.

(a) Type II Familial Dysautonomia (Familial Sensory Neuropathy with Anhydrosis). In 1970, Wolfe and Henkin reported their observations in two patients suffering from familial dysautonomia. In contrast to the other patients with Riley-Day Syndrome discussed earlier, these two patients have normal papilla and taste buds in their oral cavities and show no sign of abnormalities in the oral region.

However, neither patient was able to detect a saturated solution of sodium chloride, sucrose, or urea, or a solution of 0.5 N hydrochloric acid as different from water; or to recognise these solutions as salty, sweet, bitter, or sour respectively. Subcutaneous and intravenous administration of

methacholine did not alter taste thresholds for any quality. The mechanism of the taste defect in this type of patient is unknown. On the basis of this taste response difference, Wolf and Henkin (1970) classified these patients as having type II familial dysautonomia to distinguish them from patients with type I familial dysautonomia (Riley-Day syndrome).

(b) Adrenal Cortical Insufficiency. Preference for salt in the food and fluids of adrenalectomized animals and humans has been known for a long time (Richter 1936, Thorn 1949). This phenomenon has been investigated again more recently, Henkin and Solomon (1962) reported an increased taste sensitivity to salt in patients with adrenal cortical insufficiency. At that stage, Henkin and Solomon hypothesized than an alteration in the volume of some body fluid compartment or in the concentration of intracellular electrolytes may be responsible for this increased sensitivity to salt.

Later, Henkin, Gill and Bartter (1963) found that patients with adrenal cortical insufficiency exhibit markedly lower thresholds for many other substances. In their study of seven cases of Addison's disease and two cases of hypopituitarism, the patients showed detection thresholds which are roughly one hundred times more acute as expressed in stimulus concentration ratios, than normal subjects in response to NaCl, KCl, $NaHCO_3$, sucrose, urea and hydrochloric acid. This increase in taste sensitivity was found to be corrected by the administration of a carbohydrate-active steroid, prednisolone. Taste thresholds were found to return to normal in every patient within the first day of prednisolone therapy and sensitivity did not increase again for three to four days after termination of therapy. Kosowicz and Pruszewicz (1967) reported similar results in their study of

seventeen patients with Addison's disease and sixteen patients with Hypopituitarism. The effect of carbohydrate-active steroids on taste is believed to be related to their role in nerve function (Henkin and Bradley 1972).

(c) Diabetes. Taste in diabetes has been investigated intermittently since the early 1900's. Hollingworth and Poffenberger (1917) reported that in diabetics, a sweet taste may be experienced in the absence of stimuli on the tongue. Fox (1932) noted that the inability to taste phenylthiocarbamide was found more frequently among diabetics than in the general population. Many researchers have now provided evidence that sugar in the blood, diabetes or added, reduces sensitivity to sweetness.

Schelling et al. (1965) compared recognition thresholds for dextrose and sodium chloride of 79 diabetic patients to those of normal subjects and reported that diabetics as a group appeared to have a higher threshold for dextrose than normals. This is not so in the case of sodium chloride, no significant difference among the two groups of subjects was observed. This abnormal threshold for sugar in diabetics has been interpreted as possibly due to frequent elevations of blood-sugar levels (Schelling et al. 1965). This view seems to be in agreement with Richter (1942) who proposed a theory that food selection, and presumably sensitivity to taste, depends on need (Amerine, Pangborn and Roessler 1965).

However, contrasting evidence has also been reported in the literature, Joergensen and Buch (1961) concluded from their study that the sense of taste in diabetes is not significantly altered. Yensen (1964) also failed to find a systematic relationship between blood-sugar levels and taste thresholds for

sucrose.

Perhaps, all the results of different studies in this area need to be reconsidered when one considers a report by Gregson (1972). He correctly pointed out that the apparently abnormal thresholds for sugar found in diabetics may be complicated by the effect of the drug regime used in treating diabetes. In his study of thirty eight diabetics, he noted:

> "Diabetics show some sensory changes, but what is a consequence of their disease and what is a consequence of their drug regime has not been disentangled",
>
> and
>
> "There is some slight average threshold elevation, but it is slight and without knowing the individual history of a patient it is not clear that, with any confidence, you could estimate his sensory loss until it was quite marked".

Therefore, until the effect of different drugs used in the treatment of diabetes on taste processes, if any, is clearly established, it is not possible to determine the effect of diabetes itself on taste processes.

(d) Patients under Treatment with D-Penicillamine. The possible effect of drugs used in the treatment of various diseases on taste sensitivity has been demonstrated in the case of D-penicillamine. Either as a hydrolytic product of penicillin; cuprimine, D-penamine or as a synthetically produced compound, Sulredox, D-Penicillamine has been used to treat various illnesses, Wilson's disease, cystinuria, rheumatoid arthritis, scleroderma, idiopathic pulmonary fibrosis and lead poisoning.

Keiser et al. (1968) reported that among twenty patients being treated with penicillamine for scleroderma or cystinuria, seven complained of subjective loss of taste for salt and sweet. The onset of this symptom occurred within three to six weeks after the commencement of penicillamine therapy. When objectively

tested for their sensitivity, median detection thresholds for sodium chloride and sucrose were approximately twelve times those in normal subjects, median recognition thresholds for salt and sweet were five times those in normal subjects. For hydrochloric acid and urea, the detection and recognition thresholds were also raised.

Similar results were found among another group of patients (Henkin et al. 1967). During treatment with D-Penicillamine, four out of a hundred patients with Wilson's disease and twenty three out of seventy three patients with scleroderma, cystinuria, rheumatoid arthritis and idiopathic pulmonary fibrosis experienced a decrease in taste acuity. This symptom has now been linked with low levels of serum ceruloplasmin or copper. Four patients without Wilson's disease who received copper as dietary supplement showed normal taste acuity in all modalities within four weeks of copper supplementation. This finding will be discussed in more detail later in this thesis.

(e) Other Diseases. Disturbances in gustatory functioning have been reported in several other groups of patients suffering from assorted ailments. Smith (1972) reported significantly lower thresholds to quinine among drug addicts and significantly higher thresholds to the same substance among alcoholics, when compared with matched control healthy subjects. The investigator interpreted the results from alcoholics as supporting the assertion that prolonged consumption of alcohol impairs taste sensation. No other evidence of taste functioning in alcoholics and drug addicts have been reported in the literature.

In the case of cystic fibrosis, an inherited disease of the exocrine glands which affects the respiratory system, pancreas

and sweat glands, contrasting evidence of taste abnormalities has been reported. Henkin and Powell (1962) found that patients with cystic fibrosis had markedly greater taste sensitivity (100 times greater) than normal subjects. Wotman et al. (1964) in an attempt to confirm this result, failed to find any abnormality in taste functioning among his group of patients with cystic fibrosis. He noted that most of his subjects had approximately normal sensitivity and that a few were hyposensitive. More recently, Hertz et al. (1975) reported evidence in support of Wotman et al.'s results. They found no significant difference in detection thresholds for sodium chloride between patients with cystic fibrosis and normal subjects. However, Hertz et al. (1975) suggested that hypersensitivity to taste stimuli as reported by Henkin and Powell (1962) could have been the result of adaptation effect of sodium chloride in the saliva of cystic fibrosis patients whose saliva sodium levels has been shown to be approximately three times those of normal subjects.

Hypogeusia, decreased taste acuity has been reported in patients with Sjogren's syndrome (Henkin et al. 1972), thermal burn (Cohen et al. 1973), respiratory infection (Henkin 1972), head trauma (Schechter and Henkin 1974) and facial hypoplasia (Henkin, Christiansen and Bosma (1970).

(f) Idiopathic Hypogeusia. Apart from those patients suffering from different illnesses causing alterations in their taste processes, Henkin et al. (1971) reported a large number of patients who exhibited decreased taste acuity (hypogeusia) without any obvious underlying cause. Many of these patients often complain that in addition to hypogeusia, many foods have an abhorrent or inappropriate taste (dysgeusia), that they have decreased smell acuity (hyposmia) and that many odorants have an

abhorrent or inappropriate smell (dysosmia). These investigators have termed this syndrome "idiopathic hypogeusia with dysgeusia, hyposmia and dysosmia".

Of the 103 patients first reported in 1972 (Schechter et al. 1972), 57 per cent reported that their symptoms began during or shortly after an upper respiratory illness, at the time commonly known as "Hong Kong 'flu", 33 per cent reported no illness or unusual event preceeding their sensory loss and the rest (10 per cent) reported that the symptoms appeared immediately following various surgical procedures unrelated to the head or neck. Detailed clinical evaluations did not reveal any abnormality in the nasal, oral or pharyngeal areas.

The aetiology of this disease is not yet clearly understood. It has been attributed to the abnormality of trace metals zinc and copper metabolism. There is evidence that these patients can be treated successfully by the administration of zinc medication (Schechter et al. 1972). The significance of this finding will be discussed in the next chapter.

The realisation that several disease states affect taste acuity and the success of some treatment programmes in correcting taste abnormalities has led to several new interpretations in the field of taste. The role of physiological, anatomical and biochemical factors in taste processes has been recently reassessed. Some of these new ideas will be discussed in the next chapter.

III. SUMMARY

In this chapter, a number of factors which affect taste perception both in normal and abnormal circumstances have been briefly discussed. The aim of this review is to provide

background information on which the proposed studies and discussions will be based.

CHAPTER IV

TASTE SENSITIVITY AND ZINC METABOLISM

A new era of taste research began with the findings on treatment of taste abnormalities. Previously, taste abnormalities have been considered either as a genetical phenomenon as in the case of response to caffeine, or phenylthiocarbamide and related chemicals, or a physiological and anatomical defect as in the case of damages to the receptors in the oral area or damages to the chorda tympani nerve. From this viewpoint, taste abnormalities cannot be corrected and attempts at treatment consequently were not made.

Since the discovery that there are patients whose taste perception is altered while suffering from various illnesses or undergoing treatment with certain drugs, taste has been considered from a different point of view. This new look at taste takes into account biochemical and metabolic factors. Taste abnormality is now thought of as a result of changes in the metabolic balances of certain elements and chemicals commonly found in the human body. With this view, it is believed that treatment of taste abnormalities is possible. Many treatment procedures employing different chemical compounds have been attempted with varying degrees of success.

In this chapter, all the treatments of taste abnormalities will be discussed in some detail. A new concept of taste functioning based on the hypotheses derived from results of these treatment programmes will also be discussed.

I. TYPE ONE FAMILIAL DYSAUTONOMIA AND METHACHOLINE

As mentioned earlier, patients with type I familial dysautonomia (Riley-Day Syndrome) have decreased taste acuity (hypogeusia) or complete absence of taste perception (ageusia). At their best, some patients exhibit significantly elevated detection and recognition thresholds for some of the four taste qualities (Henkin and Kopin 1964, Henkin 1967).

Biopsy specimens from the tongues of these patients showed a number of deviations from normal subjects. First, papillae and taste buds were not found. Secondly, the only neural structures found in their tongues were unmyelinated nerve fibres that did not terminate on any specialized epithelial structure. Thirdly, these unmyelinated nerve fibres were fewer in number than those found in comparable biopsy specimens taken from people with normal taste acuity. Fourthly, there was no increase in number of unmyelinated nerve fibres over the anterior two thirds compared with the posterior one third of the tongue, although this is the case in normal individuals (Henkin 1969).

From these light and electron microscopic studies, it would have been acceptable to assume that the decrease in taste sensitivity was due to the absence of the taste buds and the defects in the papillae. However, it was found that parenteral administration of the drug methacholine, besides producing tears in these patients (Kroop 1956), also improved their taste sensitivity (Henkin and Kopin 1964). Following the administration of methacholine, the abnormally elevated detection and recognition thresholds for sodium chloride and sucrose were lowered. The detection and recognition thresholds for these two substances were within normal levels during the period while spontaneous tearing occured. Within thirty minutes after the injection, when the

effects of the drug wore off, the patients became quantitatively less able to detect or recognise either the salt or sweet taste.

With the above finding, it became clear that even without both lingual papillae and taste buds, taste perception is still possible with the presence of the unmyelinated nerve fibres alone. Henkin, Graziadei and Bradley (1969) while reviewing this result, suggested that taste buds merely play the role of chemical sieves, providing pores of small, controlled size through which chemical stimuli may reach the termination of the unmyelinated nerve fibres, "free nerve endings", normally found in the taste buds. Once this contact is made, a nerve can be depolarized resulting in taste responses.

What happens in the case of patients with Riley-Day Syndrome seems to be as follows, due to the absence of lingual papillae and taste buds, and because the unmyelinated nerve fibres do not terminate on any epithelial structure, the taste nerves are not in direct contact with the taste stimuli. A chemical is needed to induce chemosensation by creating pores in the epithelium allowing the taste stimuli to reach the unmyelinated nerve fibres of the tongue. Parenteral methacholine is apparently effective since it is associated with a general increase in membrane permeability including that of the lingual surface. Topical acetylcholine or methacholine placed on the tongue of these patients did not alter taste acuity (Henkin 1969) because topical application does not have the same effect on membrane permeability as parenteral administration of the drugs. Once the effects of the drug wear off, the membrane permeability returns to the previous level, preventing direct contact between the stimulus and the nerve endings, hence the return to the abnormal level of taste acuity.

II. ADRENAL CORTICAL INSUFFICIENCY, CARBOHYDRATE ACTIVE STEROIDS (CAS) AND TASTE

There had been a large number of studies done on the gustatory functioning of patients with adrenal cortical insufficiency (ACI). Henkin and Solomon (1962) recorded that ten patients with untreated or inadequately treated adrenal or pituitary insufficiency had significantly decreased detection thresholds for salt.

The range of taste stimuli was extended in a study by Henkin, Gill and Bartter (1964) who reported the observation of nine patients who exhibited symptoms of adrenal cortical insufficiency from different diseases including idiopathic adrenal cortical insufficiency (Addison's disease), patients who have had both adrenal glands removed by surgery and patients with panhypopituitarism. They found that among these patients, detection thresholds for the taste of potassium chloride, sodium bicarbonate and sodium chloride (salt), sucrose (sweet), urea (bitter) and hydrochloric acid (sour) were significantly decreased. Expressing the detection thresholds for these substances in concentration (mmoles per litre) units, the untreated patients detected each of the compounds at about one one-hundredth the concentration detected by normal subjects. Recognition thresholds for these compounds, however, were found to be significantly elevated. In other words, patients with untreated adrenal cortical insufficiency showed increased detection acuity and decreased recognition acuity. These results are similar to those obtained by other investigators (Kosowicz and Pruszewicz 1967).

It is well established that untreated, these patients have abnormally high levels of serum potassium concentration and low levels of serum sodium concentration with defective extracellular

fluid volume control. These factors were first thought to affect taste acuity. In a series of experiments where 20 mg of deoxycorticosterone acetate (DOCA) was administered to the patients with ACI for a period of two to seven days, the serum sodium and potassium levels were brought to normal without any significant change in the abnormally low detection thresholds and high recognition thresholds for the four taste modalities (Henkin et al. 1963, Henkin 1970, Henkin and Bradley 1972). DOCA is a potent sodium-potassium active steroid generally used in replacement therapy among ACI patients.

In the same series of experiments, changes in taste detection acuity were studied in ACI patients being treated with prednisolone, a carbohydrate active steroid (CAS), 20 mg a day for two or more days. This treatment was found to return taste thresholds to normal in all taste modalities. During treatment with prednisolone, the abnormal serum levels of potassium and sodium remained unchanged. This return to normal of both detection and recognition thresholds is independent of the type of carbohydrate-active steroid used. In different experiments, prednisolone, hydrocortisone, cortisone and dexamethasone have all returned taste acuity to normal. The success of the treatment is also independent of the route of administration of the hormone; oral, subcutaneous, or intravenous administration produces the same result. However, the amount of hormone administered does influence the time required to return taste acuity to normal (Henkin 1970). After administration of a large amount of hormone, less time is required to return taste acuity to normal than after administration of a small amount of hormone.

The effectiveness of CAS treatment has further been demonstrated in another series of experiments involving the

stopping and starting of the hormone replacement therapy and the observation of detection thresholds for the four modalities. An ACI patient whose detection thresholds were significantly decreased was treated orally with 20 mg prednisolone daily. Her detection threshold for sodium chloride returned to normal within forty eight hours of therapy. Taste acuity remained within normal limits during ten days of prednisolone therapy. Four days after termination of therapy, the patient's taste acuity returned to the abnormally high level shown before commencement of therapy. Six days after treatment with prednisolone was discontinued, the patient was given DOCA 20 mg intravenously each day for two days. This treatment had no effect on taste acuity. On day 24, 200 mg of hydrocortisone acetate, another CAS was given intravenously and taste detection thresholds for all taste qualities returned to normal levels within twenty four hours (Henkin 1970).

Normal volunteers given large amounts of the sodium-potassium active steroids parenterally in the form of 20 mg DOCA for two to five days, did not show any significant changes in taste acuity. These subjects were also given large amounts of CAS orally as 50 mg of prednisolone for five days and no change in taste acuity was noted (Henkin 1972).

Since the absence of CAS among patients with ACI has been shown to produce increased detection acuity, one may expect decreased detection acuity among patients with excessive amounts of CAS. This in fact has been shown to be so. Patients with excessive endogenous levels of CAS e.g. the cases of Cushing's syndrome, adrenal cortical hyperplasia, adrenoma or carcinoma, exhibit both increased detection and recognition thresholds (Henkin 1970). In these patients, the detection and recognition threshold concentrations are usually the same. Henkin and Bradley

(1972) reported that suppression of endogenous CAS secretion by administration of exogenous CAS (decadron suppression), SU 4885, aminoglutethamide, or following adrenalectomy, returned taste acuity of these patients to normal as long as suppression was maintained. On the other hand, treatment with sodium-potassium active steroids did not alter taste acuity among these patients.

From this data, we can conclude that absence of CAS, as in the case of adrenal cortical insufficiency, produces a significant decrease in detection thresholds and a significant increase in recognition thresholds for all taste qualities. Excessive endogenous CAS levels, as in the case of Cushing's syndrome, results in significant elevations in both detection and recognition thresholds for all taste modalities. Therefore, it seems that the perception of taste is dependent upon changes in CAS concentration in man.

The question that remains is, how does CAS affect gustatory functioning?

In an attempt to answer this and similar questions, Henkin Graziadei and Bradley (1969) separated taste process into two sets of events, preneural and neural. The preneural event, presumably takes place at the outer membrane of the taste receptor, involving the interaction between tastant and the receptor molecule. The result of this specific reaction at this level is most likely the formation of a tastant-receptor complex (Henkin and Bradley 1972). This complex formation, then produces the signal which initiates the neural events.

The neural events of taste involves the depolarisation of the taste nerves subsequent to the preneural events. How the formation of the tastant-receptor complex leads to the depolarisation is not known. However, Henkin and Bradley (1972)

suggest that there is an anatomical separation between the outer membrane of the taste receptor, where the tastant-receptor complex forms and the junction at which the taste nerve joins the receptor. This separation point presumably is the recently identified taste pore. The neural events also include conduction of the impulse along the taste axons, transmission across the synapses of the taste system and integration of the taste information by the central nervous system.

In the case of carbohydrate active steroids, Henkin and Bradley (1972) hypothesize that CAS deficiency and excess states may affect the preneural events of taste by altering the binding constants by which tastant-receptor molecule complexes occur or by affecting the stoichiometry of binding. However, this hypothesis is based on visual fitting of a hypothesized equation onto the data obtained during CAS treatment and yet to be substantiated by biochemical demonstrations.

Henkin and Bradley's explanation of the role of CAS at the neural events of taste seems more plausible. Essentially, they suggest that CAS may inhibit taste acuity through the control of neural excitability and of timing by which impulses traverse axons and synapses in the human nervous system. It is generally accepted that in the absence of CAS there are significant changes in the manner by which neural signals are conducted along axons and transmitted across synapses (Henkin et al. 1969, Henkin et al. 1967, Henkin and Ojemann 1967). In CAS deficiency states, axonal conduction velocities are increased slightly while synaptic delays are markedly increased. These changes in neural conduction and transmission in CAS excess or dificiency states result in gross abnormalities in the timed arrival of sensory signals in the central nervous system from the periphery. Since sensory recognition is

dependent upon the timed arrival of sensory signals in the central nervous system in a specific manner, alterations in this timing would result in a significant information loss. This, Henkin and Bradley (1972) believe, explains the inability of the patients to recognise tastants at normal recognition threshold concentrations. They suggest further that increased tastant concentrations are required for the patients to recognise the appropriate perception of taste because this increase in concentration will increase the number of afferent signals per unit time which will overcome this information loss.

The increase in detection acuity in adrenal cortical insufficiency patients is attributed to the absence of CAS. Because of this absence, the inhibition of some of the neural events of taste are removed and increased neural excitability occurs. This leads to the increase in detection acuity (Henkin, Graziadei and Bradley 1969). Furthermore, CAS may also play a significant role in controlling the base line or background level of neural activity. The absence of CAS may lower the background level of neural activity allowing incoming signals of low intensity to be more easily detected because of their higher signal to noise ratio.

At this stage, the available data seems to indicate that CAS plays an important role in taste processes with changes in CAS levels resulting in alterations in taste acuity. The role which CAS plays in taste processes is not yet clearly established, further experimentation may clarify the position at both the preneural and neural events.

III. PENICILLAMINE, THIOLS, COPPER AND TASTE

It has been mentioned in Chapter III of this thesis that a period of treatment with penicillamine produced significant decrease

in taste acuity among patients with different illnesses. We will look at this phenomenon in more detail at this point.

(1) Penicillamine and Taste

Penicillamine, either as a hydrolytic product of penicillin ('cuprimine', 'D-penamine') or as a synthetically produced compound ('sulredox') has been used to treat various illnesses including Wilson's disease, cystinuria, rheumatoid arthritis, scleroderma, idiopathic pulmonary fibrosis and lead poisoning (Henkin et al. 1967). In a preliminary study on eleven patients with scleroderma and nine patients with cystinuria, Keiser et al. (1968) found that four of the patients with scleroderma and three of the patients with cystinuria reported abnormalities of taste. This is an overall incidence of 35 per cent. These patients subjectively reported reduction in sensitivity to salt and sugar within three to six weeks after therapy with penicillamine was started. Upon objective testing, these patients showed significant increase in both detection and recognition thresholds for all four taste modalities. These taste alterations persist without deterioration as long as penicillamine therapy was continued. Taste acuity among these patients did not return to normal until four to six weeks after the discontinuation of the therapy. Detailed anatomical investigation did not find any anatomical impairment in the oral region of those patients who exhibited symptoms of hypogeusia except for one patient whose biopsy specimen did not reveal any taste bud. At this stage penicillamine therapy was considered wholely responsible for this alteration in taste since Henkin (unpublished observation) noted that neither subjective nor objective taste abnormalities have been found in untreated patients with scleroderma or cystinuria.

(2) Penicillamine, Copper and Taste

Henkin et al. (1967) confirmed the above results in another experiment. Their subjects included twelve patients with scleroderma, twelve with cystinuria, forty four with rheumatoid arthritis, five with idiopathic pulmonary fibrosis and one hundred with Wilson's disease. Among these patients, twenty three out of seventy three non-Wilson's disease patients (32 per cent), showed decreased taste acuity for all taste modalities in contrast to four out of one hundred (4 per cent) of patients with Wilson's disease who exhibited hypogeusia. The common picture among these patients is the same as the above mentioned study, that is patients report subjective decrease in sensitivity to salt and sugar within approximately four to six weeks after the initiation of D-penicillamine therapy. It is noted that patients exhibit this symptom after the drug dosage was greater than 1 gm. The elevation in taste thresholds was shown upon objective testing of these patients. Hypogeusia does not deteriorate further even when the dosage of D-penicillamine is raised to 5 gm. daily but persists as long as therapy is continued. Taste acuity returns to normal four to eight weeks after the termination of therapy. In this study, it was noted that during penicillamine therapy, serum ceruloplasmin and serum copper concentration was decreased greatly among the non-Wilson's disease patients raising the possibility of copper participating in the taste process of these patients.

To investigate this possibility further, copper, either as dietary supplement or as copper sulphate was administered to the patients with hypogeusia while continuing D-penicillamine therapy at a dosage of 2 gm daily. Following this supplement, serum ceruloplasmin levels returned to normal within four to eight days and objective taste acuity returned to normal in each of the

patients within four weeks.

From this result, Henkin et al. (1967) concluded that there is a possibility that copper plays a role in the physiology of taste because of the following indications:

(a) decreases in taste acuity were associated with decreases in serum ceruloplasmin levels which indicates copper deficiency except in patients with Wilson's disease.

(b) patients with Wilson's disease have increased concentration of tissue copper (Sternlieb 1966) which is not reduced to normal with prolonged treatment with penicillamine. This seems consistent with the observation that there is a markedly lowered incidence of hypogeusia among them.

(c) The improvement in taste acuity upon treatment of hypogeusic patients with copper can be associated with parallel increases in serum-ceruloplasmin to normal levels.

These experiments in man were repeated in rats (Henkin, Keiser and Kare 1968; Kare and Henkin 1969). Administration of D-penicillamine produced hypogeusia as indicated by elevated preference thresholds for salt and sugar. Administration of copper with continued administration of the drug returned taste acuity to normal levels.

(3) Penicillamine, Thiols, Copper and Taste

From the above indications, it seems that Penicillamine and copper affect taste acuity in a rather straight-forward relationship, but this relationship becomes complicated when one considers the case of patients with rheumatoid arthritis.

It has been established that some patients with rheumatoid arthritis have elevated serum concentrations of copper and ceruloplasmin, and usually an abnormally low serum thiols (RSH) concentration (Henkin and Bradley 1969). After treatment with

D-penicillamine, these patients exhibited serum copper concentrations that were lower than normals (Henkin et al. 1967) and increased serum RSH concentrations (Lorber 1966). Therefore, administration of D-penicillamine adds thiols to the serum and removes copper from it. Under the conditions of lower serum copper and increased serum RSH concentration, some of these patients showed the symptom of hypogeusia (Henkin et al. 1967). This raises the suggestion that thiols as well as copper play a role in taste processes (Henkin and Bradley 1969).

The opportunity to investigate this possibility arose when a patient with multiple myeloma of a rare G3 subclass reported symptoms of hypogeusia (Henkin and Bradley 1969, Henkin, Graziadei and Bradley 1969). This patient had grossly elevated detection and recognition thresholds for all taste qualities. Her serum copper concentration was within normal limits while her exchangeable thiol concentration (RSH) level was assumed to be elevated. In order to test their hypothesis that thiol does affect taste sensitivity, Henkin and Bradley (1969) administered copper in the form of oral copper sulphate to the patient with an aim to remove her exchangeable thiol concentration by chelation or complex formation with copper. They found that after four days of daily administration of 20 mg copper ions, the patient's taste acuity for each taste quality increased from extremely low levels to the normal range, and after eight days of treatment her acuity was not different from the normal subjects in any way, both subjectively and objectively. During therapy, her serum copper concentration increased. After the copper therapy was stopped, hypogeusia returned within forty eight days.

This result was taken as indicating that copper was effective in correcting taste abnormality because of it's function in tying

up excessive, exchangeable RSH in this patient. Similar results were obtained when Henkin and Bradley (1970) administered two other transition metals known for high association constants with thiols to this patient in separate experiments. These two metals were zinc and nickel given in the forms of zinc chloride and nickel acetate respectively. Both metals were successful in returning her hypogeusia to normal for each taste quality. Termination of each metal treatment was followed by the return to previous symptoms of hypogeusia.

The above data raises the possibility that thiol and copper interaction may be responsible for the changes in taste acuity among patients undergoing treatment with penicillamine, a well known thiol containing drug. This possibility was investigated in an experiment using four groups of rats employing preference thresholds to sodium chloride to indicate changes in taste acuity (Henkin and Bradley 1969). The results showed that rats treated with D-penicillamine or 5 mercaptopyridoxal, another thiol containing drugs showed hypogeusia. The thiol containing drugs produced hypogeusia without affecting total copper levels.

These results seem to indicate that excessive levels of exchangeable thiol concentration lead to hypogeusia which can be corrected by the administration of transition metals capable of chelating with thiols.

(4) The Role of Thiols and Metals in Taste Process

In an attempt at explaining the role of thiols and metals in taste process, Henkin and Bradley (1969) hypothesize that thiols and metals are involved in a reaction which regulates the preneural events of taste. They assume that thiols have inhibitory influence on taste processes. This assumption is based on the observation that taste acuity decreased with thiol administration. The

reversal of thiol inhibition by copper, zinc and nickel can be explained by a hypothesis that thiols and metal ions are in near-chemical equilibrium with one another in the body through a series of reactions including a complex formation of the type

$$2\ RSH + Me^{++} \rightleftharpoons (RS)_2\ Me + 2H^{+}$$

where RSH and Me^{++} represents thiols and metal ions respectively.

If thiol concentration is increased, the equilibrium is shifted to the right, and taste acuity decreases. This was exemplified in patients and animals who developed hypogeusia after treatment with thiol-containing drugs (Henkin, Graziadei and Bradley 1969). If metal concentration is decreased, the equilibrium is also shifted to the right and taste acuity decreases. This is the case where the patients and animals developed hypogeusia after treatment with drugs which removed copper from the organism.

The manner in which this chemical equilibrium affects taste perception is hypothesized to be at the preneural level. The taste bud has been shown, with the aid of the scanning electron microscope, to consist of a large pore and a membrane in a surrounding papilla. In the preneural event of taste, the tastant enters the pore and diffuses down its length or through its lateral surfaces. Henkin and Bradley (1969) postulated that there exists a "gate-keeper protein" at the taste pore which are protein molecules involved in the regulation of the amount of tastant which passes through the taste bud pore per unit time. They further postulated that thiol and metal equilibrium regulates the size of this gate-keeper protein molecule by binding with it producing a conformational shift of the protein molecules which in turn control the diameter and permeability of the pore and its membrane. They said:

"If the gate-keeper protein reduces the pore diameter and membrane permeability to a value smaller than normal following

administration of a thiol-containing drug, detection thresholds would be elevated because a greater concentration of tastant would be needed to raise the net flux through the pore and its membrane to a level which would initiate the neural events. If metals such as copper or zinc were administered concomitantly, the gate-keeper protein would return toward its normal conformation, increase the pore size and membrane permeability, and reduce the concentration of tastant required to trigger the neural events. Conformational changes of the gate-keeper protein would produce parallel changes in threshold for each of the four qualities of taste, as observed."

According to Henkin and his colleagues, it seems that taste acuity is inhibited at the preneural level by thiol containing drugs while carbohydrate active steroids exert inhibitory influence on the neural events of taste.

IV. ZINC METABOLISM AND TASTE

In 1971, Henkin et al. reported a new syndrome, idiopathic hypogeusia (decreased taste acuity) with dysgeusia (distorted and perverted taste perception), hyposmia (decreased smell acuity) and dysosmia (distorted and perverted smell perception). Among the thirty five patients reported in this study, hypogeusia was found in each patient but dysgeusia, hyposmia and dysosmia were not uniformly present. These abnormalities in taste and smell were apparently without any obvious underlying cause. The patients also complained of other symptoms which occured with variable frequency. These included persistent sensations of saltiness, sweetness, sourness, bitterness or metallic tastes in the oral area in the absence of food, which could not be relieved; sensations of a persistent foul odor in the nasopharynx which also could not be relieved; and vertigo, hearing loss, loss of libido and unexplained hypertension.

Of these thirty five patients, eighteen (51 per cent) had upper respiratory tract illness directly preceeding or concomitant with the onset of hypogeusia, two (6 per cent) developed hypogeusia following surgical treatment and the rest reported no specific

event prior to the onset of symptoms. Their median detection and recognition thresholds were significantly elevated above normal. By definition, hypogeusia in this syndrome represents an elevation of detection and/or recognition threshold for at least one taste quality. Without providing details, Henkin et al. (1971) reported that treatment with oral zinc ion was successful in correcting many of the symptoms of this syndrome as well as improving taste thresholds. This treatment was apparently given following the success of transition metals in correcting hypogeusia resulting from the administration of thiol containing drugs. Zinc was chosen in preference to copper or nickel because it is the least toxic and best tolerated member of the transition metals.

Another important point in this report is that, electron microscopic examinations of the circumvallate papillae of these patients revealed ultrastructural changes in cellular organisation in the taste buds compared to normal subjects. Henkin et al. (1971) reported:

> "There was a general lack of organisation of the pore area with a loss of the normal fine cellular projections, decreases in number and in the normal stratification of neurosecretory granules, and an absence of the normally appearing dense extracellular material. There was a generalised vesiculation and vacuolisation of the cytoplasm of two cell types; cellular differentiation and organisation is much less than in normal buds. In some cells the fibrillar material which normally appears near the nucleus disappeared and was replaced by clear glassy areas."

However, the relevance of these structural changes in the taste buds to the alterations in taste acuity has not been elucidated.

By 1972, over 4000 patients with this syndrome had been identified by Henkin and his co-workers at the National Heart and Lung Institute, Bethesda, Maryland, U.S.A. One hundred and three of these patients were observed in a single blind study (Schechter et al. 1972). They were 48 men and 55 women aged between 25 to 81 years (mean age 55 years). Their medical history prior to the

onset of the symptoms and their symptoms were similar to the group mentioned above. Ninety three per cent of these patients (96 out of 103) had hypogeusia, the remaining seven complained either of dysgeusia, hyposmia or dysosmia.

Extensive clinical evaluation and laboratory tests were conducted but failed to relate the hypogeusia to any metabolic, neurological or drug induced abnormality among the patients. Serum concentrations of zinc and copper and twenty four hour urinary excretion of these trace metals were determined in ninety one patients with hypogeusia and in ninety five normal volunteers. It was found that the mean total serum zinc concentration of the patients was significantly lower than that of control subjects ($p < .001$) while urinary zinc excretion was slightly but not significantly higher. Mean total serum copper concentration was significantly elevated above normal among the patients ($p < .001$). There was no significant difference in urinary copper excretion between the patients with hypogeusia and control subjects.

Following the diagnosis of hypogeusia, the patients were assigned to a single blind study. The experimental design was such that the patients were first treated with placebo in gelatin capsules orally four times daily with food until the following criteria were met.

(1) No change in taste acuity both objectively and subjectively had occurred.

(2) Subjective or objective changes which did occur remained the same for two consecutive measurement periods.

When these criteria had been met, the patients were then treated with zinc ion in the form of zinc sulphate in clear gelatin capsules given four times daily with food according to two dosage schedules.

(1) 25 mg per day as Zn^{++} (6.25 mg q.i.d.)

or (2) 100 mg per day as Zn^{++} (25 mg q.i.d.)

In group (1) if no subjective change in taste or objective change in taste thresholds was evident after two to four months, the dosage was increased to 50 mg per day. If this dose failed to alter taste acuity subjectively or objectively in two to four months it was increased to 100 mg per day.

However, this report of Schechter et al. did not provide a clear indication of the number of patients involved in each treatment group, making an evaluation of the treatment results a confusing and difficult task. Out of 103 patients first mentioned in this report, 47 were treated with placebos. Following that treatment, 42 patients, presumably out of the original 47 patients were given zinc ion treatment. There was no mention of the number of patients in each zinc dosage schedule. The number of patients who received zinc ion at the dosage of 100 mg Zn^{++} per day shown in a table ranged from 10 to 20 patients.

The result of treatment both by placebo and zinc ion was reported as follows:

"Before and after placebo administration, differences in the eight thresholds measured (four detection, four recognition) were not statistically significant, i.e. placebo did not significantly lower any of the thresholds ($p > .15$, 1 -sided t - test). After the smallest dose of zinc given (25 mg/day) five of eight thresholds (salt recognition, sour and bitter detection and recognition) were significantly decreased ($p < .05$). After the largest zinc dose given (100 mg/day) significant decreases were noted in all thresholds ($p < .05$). The dose of 50 mg/day was given only to those patients who did not respond after 25 mg/day and it produced decreases in only two thresholds, the recognition of salt and sour."

Of the patients treated with 100 mg/day Zn^{++}, Schechter et al. (1972) said:

"Sixty per cent of the patients had a restoration to normal (within normal median limits) of thresholds for sweet detection and recognition, 67% experienced a return to normal for salt detection, 40-50% for salt recognition, sour detection, and bitter detection and recognition, and 25% for sour recognition."

This report did not mention the length of time taken before the treatment returned the patient's taste acuity to normal, nor did it report any change in zinc serum and urinary excretion levels during and at the end of treatment with zinc medication. These inadequacies prevent readers of the report from making any meaningful assessment of the zinc treatment in connection with hypogeusia.

Schechter and his colleagues concluded that the oral administration of zinc in doses of 100 mg/day produced significant improvement in taste acuity in patients with idiopathic hypogeusia. Smaller doses of oral zinc also produced significant improvement in taste acuity but in fewer taste qualities. They noted however, that it was not possible to predict which patients would improve on zinc therapy. No correlation could be made between etiology of the syndrome, duration of symptoms, initial serum and urinary metal concentrations and the effectiveness of zinc. From the above results, they noted that zinc and copper metabolism played a role in taste processes but the exact nature of the relationship was not clear. They suggested that the decreases in serum zinc concentration and increases in serum copper concentration must be considered within the context of a complex metabolic net whose major determinants were not yet known.

Subsequent to the above study, the same group of researchers has reported results from several studies which supported the hypothesis that zinc metabolism plays an important role in taste processes.

Cohen, Schechter and Henkin (1973) reported the results from a study of nineteen patients who suffered second or third-degree thermal burns over five per cent to seventy five per cent of their body surface. Eighty four per cent or sixteen out of these

nineteen patients had hypogeusia. The hypogeusia in some of these patients was very severe; three showed decreased sensitivity in both detection and recognition of all taste qualities. Seven patients had complete loss of sensitivity for one or more taste qualities. These symptoms cannot be correlated with the extent or degree of thermal injury.

Analysis of serum zinc and copper concentrations and urinary zinc and copper excretion for twenty four hour periods was conducted both among the nineteen patients and a group of 150 control subjects. There was a significant decrease in serum concentration of total zinc ($p<.001$) and an increase in urinary excretion of zinc ($p<.01$) in patients with thermal injury and hypogeusia when compared with control subjects. Patients with thermal injury and hypogeusia also exhibited significantly greater urinary excretion of zinc compared to patients with thermal injury without hypogeusia ($p<.02$). The serum concentrations of copper did not differ in patients with thermal burns when compared with control subjects. Urinary excretion of copper was slightly greater among the patients than the controls but this is not statistically significant ($p>.05$).

These results were considered in support of a relationship between hypogeusia and altered zinc metabolism. There was no attempt at treating the patients with hypogeusia with zinc medication.

Further strong evidence was reported by Henkin, Keiser and Bronzert (1972). In this experiment, five patients with scleroderma and three normal volunteers were given the amino acid L-histidine at different doses over a period of two days. L-histidine normally produces a zinc loss in humans resulting in decreased serum zinc concentration and increased urinary excretion

of zinc. This was shown to be so in both patients with scleroderma and the normal subjects. Administration of 32 g. or higher doses of histidine produced elevations of 5 to 80 fold in thresholds for sodium chloride, sucrose, hydrochloric acid and urea among patients with scleroderma. Similar elevations in thresholds but to a lesser degree were observed among the normal subjects. Withdrawal of histidine was followed by the return to normal of serum zinc concentration and urinary zinc excretion and the disappearance of hypogeusia. The administration of 100 mg/day of zinc ion to the subjects being given 64 g. of histidine also returned hypogeusia to normal within eight hours.

Similar results have been demonstrated among rats fed a zinc deficient diet (McConnell and Henkin 1974). Taking preference for sodium chloride among rats as an indication of taste sensitivity, it was shown that rats exhibit taste alterations within three days of the initiation of zinc deficient diet with symptoms of anorexia within the first two days of the diet. Plasma zinc concentrations and six hour zinc urinary excretion were lower among rats fed zinc deficient diet when compared with control subjects.

Furthermore, this association between alterations in zinc metabolism as indicated by decreased serum or plasma zinc concentrations with or without increased urinary zinc excretion, and the occurance of hypogeusia has been demonstrated in several other patient groups. These include various forms of liver diseases (Henkin and Smith 1971, Henkin and Smith 1972), growth retardation (Hambridge et al. 1972) and in patients after head trauma (Schechter and Henkin 1974). However, none of these studies has employed treatment with zinc ion medication.

So far, this review has not considered hyposmia, the symptom of decreased smell acuity, which is commonly seen in

association with hypogeusia. In their first report of a single blind study with zinc therapy, Schechter et al. (1972) reported that eighty five per cent of patients with hypogeusia also complained of hyposmia. This symptom was also cured with the administration of zinc medication, suggesting a relationship between zinc metabolism and alterations in smell acuity. This relationship has been demonstrated in many subsequent reports. These include, among patients being treated with histidine (Henkin, Keiser and Bronzert 1972), patients with viral hepatitis (Henkin and Smith 1971) and among patients after head trauma (Schechter and Henkin 1974).

The various studies which have been cited above suggest that deficiencies of zinc are involved in the pathogenesis of hypogeusia and in some cases, hyposmia. However, the pathogenetic mechanisms involved still remain obscure.

The logic behind the administration of zinc ion in the treatment of hypogeusia in the first instance is due to its lowest toxicity among the transition elements which have been previously demonstrated to correct hypogeusia among patients treated with penicillamine.

When it was found that patients with idiopathic hypogeusia had decreased serum zinc concentration and increased serum copper concentration and that zinc returned hypogeusia to normal, a complex metabolic net involving zinc and copper was suggested to play a role in taste processes. The mutual antagonistic properties of zinc and copper levels in the blood have been mentioned earlier in this thesis, i.e. that it is common to find high serum copper concentration associated with low serum zinc concentration in the blood (Cartwright and Wintrobe 1964, Vallee 1959, Spencer et al. 1965). It is possible that zinc metabolism alone is responsible

for taste alterations and that changes in copper levels in the blood are due to changes in zinc metabolism. However, copper was not considered further when no relationship with hypogeusia was found among patients with thermal burns.

Following the finding that rats fed a zinc deficient diet exhibit hypogeusia and anorexia with associated decreased serum zinc concentration and increased urinary zinc excretion, McConnell and Henkin (1974) postulated that zinc plays a role in taste processes through its protein synthesis activities. It has been shown that zinc is involved as a cofactor in DNA - dependent RNA polymerase activity and hence protein synthesis. This suggests that it may play a major role in the metabolism of any cellular system that is undergoing rapid turnover. Cells of the taste buds have been demonstrated to turn over rapidly (Beidler and Smallman 1965). Thus, the requirement for zinc by cells of the taste buds may be expected to be high and any process that interferes with the availability of zinc might result in malfunctioning of the taste buds. This hypothesis is yet to be demonstrated biochemically.

Another hypothesis advanced in an attempt to explain the role of zinc in gustation has been attributed to Henkin (Culliton 1975). This hypothesis suggests that there is a zinc containing protein that is a constituent of normal saliva and that this protein plays a role in the growth and nutrition of the taste buds. In proving this hypothesis, Henkin, Mueller and Wolf (1975) developed a technique for the estimation of zinc concentration of parotid saliva using flameless atomic absorption spectrophotometry. They also reported that there was a significant difference in the zinc content of parotid saliva of patients with idiopathic hypogeusia and that of subjects with normal taste acuity. Parotid saliva

zinc concentration in thirty four subjects with normal taste acuity was reported to be 51 ± 14 ppb (parts per billion) (mean ± 1 S.D.) while parotid saliva zinc concentrations in forty seven patients with idiopathic hypogeusia was 10 ± 6 ppb, a significantly lower value than that in normal subjects ($p<.001$).

Henkin et al. (1975) reported a successful isolation of a zinc protein from human parotid saliva of subjects with normal taste acuity. They accomplished this by the method of gel filtration and ion exchange chromatography. They assumed that this protein plays a role in taste process and named it "gustin". Although its function in taste remains to be established, Henkin and his colleagues argue that a role is implied by the fact that patients with taste disorders have lower than normal levels of salivary zinc. However, body pool of zinc has been shown to fluctuate with a number of factors, as discussed in Chapter II of this thesis. Also, there are as many as seventy two zinc containing proteins in the human body (Culliton 1975). These two points dissuade any premature conclusion that gustin has a major role in taste processes.

On the face of the evidence discussed so far, it seems likely that zinc plays a part in taste processes. However, further investigations are needed before any conclusion can be drawn concerning the mechanisms involved and the exact role that zinc plays in gustation.

Perhaps before further conclusions are drawn, it may be appropriate to take a brief critical look at the studies conducted by Henkin and his associates. This group of scientists has published a great number of reports on the subject of taste in several learned journals constituting nearly the only source of literature on taste abnormalities. All of their experiments on

taste threshold measurements employ one common method of measurement of taste acuity which they refer to as "three stimulus, forced-choice drop technique". In this technique, three drops are placed consecutively on alternate sides of the anterior one-third of the tongue. Two of the drops are distilled water, one of the drops is distilled water with a solute. The patient or subject is required to taste each drop to detect which of these drops contains a solute, and to identify the dissimilar drop as salty, sweet, sour or bitter. Various concentrations of sodium chloride, sucrose, hydrochloric acid and urea are normally used as representative of salt, sweet, sour and bitter tastes respectively. The lowest concentration of a solute which the patient consistently detects as different from water is defined as the detection threshold. The lowest concentration of a solute consistently recognised appropriately as salty, sweet, sour, or bitter is defined as the recognition threshold.

This method is a variation of the Triangular Testing method first used about 1946 (Helm and Trolle 1946). In the food industries, where variations of the triangular test have been employed in food discrimination and food preference testing, these methods have been shown to produce a bias problem. In preference testing, there is a tendency for the subjects to record a bias against the odd sample of the triangle (Schutz and Bradley 1954). Gregson (1960) found that in both preference and discrimination testings, the bias was not related to the substance tested but intrinsic to the method. The direction and magnitude of the bias were dependent on the information given to the subject about the differences he was asked to detect. He also reported that the bias can be balanced out for most practical purposes by using a balanced experimental design and taking sufficient subjects and

presentations of the samples.

More recently, methods of sequential analysis have been extended to the triangular test. The inherent variability of natural products raises some doubts about the use of this test since it may be impossible to obtain two genuine replicates (XX) to form the triads of the type XXY (Harper 1972). Even in the case of pure chemical stimuli, it is not certain that two samples of the same solution X will be perceptually identical in the XYX arrangement, due to the possible effect Y may have on the perception of the second X sample. Only a carefully balanced experimental design can minimise this problem.

Harper (1972) comments that it is often reported that the odd sample is detected more correctly than the other two and this is consistent with Helm and Trolle's original data. In his opinion, there seems to be some doubts from the published data whether all possible arrangements have been balanced out. He indicates that there are occasions when although there is a definite difference between the two types of samples, it is difficult to tell to which of the three samples the difference should be assigned.

One can gather from the available information therefore, that if the triangular test is to be used, great care must be taken to balance out all the possible arrangements of the three samples in the experimental design. Henkin and his colleagues have been criticized for using an unbalanced variation of the triangular method (Harper 1972). Their method provides an unbalanced sample arrangement in that only one of the two possible combinations of positive stimuli and blanks has been employed. This unbalanced design together with the small number of subjects involved in most of the studies of taste alterations indicate that the thresholds produced by this method may not be reliable.

Also, as already discussed previously, the drop method has been demonstrated to produce higher thresholds than methods using sips (Hinchcliffe 1958). This choice of method prevents any comparison of threshold values between those obtained in their studies and those obtained from other sources which usually employ sip methods.

In some of their studies on hypogeusia and zinc metabolism, there seems to be some inconsistencies in the definition of hypogeusia. In a study by Cohen, Schechter and Henkin (1973), patients with detection or recognition thresholds, or both, only one concentration unit above the upper limit of the normal threshold range for any taste quality, were not considered to have hypogeusia. According to this norm, a group of patients normally classified as having hypogeusia, by the original definition (Henkin et al. 1971) would be classified as "normal". This inconsistency makes it difficult to compare one study to others even by the same authors.

Another arbitary guideline used by these investigators concerns the sour-bitter confusion commonly seen among subjects in taste experiments (Gregson and Baker 1973). Cohen, Schechter and Henkin (1973) noted that confusion of the recognition of sour with bitter at concentrations of hydrochloric acid less than or equal to 30 millimoles was not considered abnormal in their study. This seems an inadequate way of dealing with the sour-bitter confusion phenomena. They did not specify whether a bitter response to a solution of hydrochloric acid at a concentration less than 30 millimoles was considered to be correct recognition of the substance.

In these experiments, no precautions have been taken to ensure consistent time of testing taste acuity. Henkin, Gill and Bartter (1963) recorded that

"Thresholds were measured by one observer in the early morning hours in the normal subjects and in the early morning hours

or evening hours or both, in the patients with adrenal insufficiency."

The time of day at which the taste acuity is measured is an important factor and considered clinically significant (Gregson 1972). Measurement of taste acuity at different times of day may produce slightly different threshold values.

Furthermore, in their study of therapy with zinc ion in the treatment of hypogeusia (Schechter et al. 1972), there is no fixed time interval between each assessment of taste acuity both prior to and during treatment with zinc ion. They said,

"Taste thresholds were determined by four experimenters and whenever possible, each patient was tested by more than one experimenter. Each patient was tested two to four times prior to any therapeutic measure and at least once during each treatment condition."

This lack of control prevents any assessment of progress of treatment as a function of time. The success of treatment seems to rely on the subjective report of normal taste perception. A great deal of information concerning recovery rate and changes in taste functioning during the treatment period is not available because the patients were not tested regularly during the four months period of treatment.

As pointed out earlier, some of their reports contain many inadequacies which make critical assessment of their results a difficult and confusing task.

Bearing these points in mind, let us now look at some conclusions that can be drawn from the experiments conducted by Henkin and his colleagues concerning taste processes.

V. A VIEW OF TASTE PROCESS; A SUMMARY

According to Henkin and his co-workers, the following conclusions based on several hypotheses have been reached.

(1) Taste processes can be divided into two sets of events.

(a) Preneural events which take place at the outer membrane of the taste receptor. These events involve the interaction between the tastant and the receptor molecule forming tastant-receptor complexes which produce the signal initiating the neural events.

(b) Neural events, which involve the depolarization of the taste nerves subsequent to the preneural events. They also include the conduction of nerve impulses along the taste axons, transmission across the synapses of the taste system and integration of the taste information by the central nervous system.

(2) Taste buds are not essential in taste processes. An important component of the process is the taste pore normally found in taste buds, which is the connection between the preneural and neural events of taste. Taste buds only act as chemical sieves holding tastant in direct contact with taste nerve endings through the taste pores.

(3) Methacholine returns taste acuity to normal among patients with Riley-Day syndrome through its effect on membrane permeability.

(4) Carbohydrate Active Steroids return taste acuity to normal in patients with adrenal cortical insufficiency by its actions at the neural events of taste.

(5) Penicillamine affects taste sensitivity at the preneural events.

(6) There are strong indications that zinc metabolism plays a part in taste processes. The exact role and the

mechanisms involved have not been established. It is suggested that a zinc containing protein plays an important role in the growth and nutrition of the taste buds.

VI. THE UNANSWERED QUESTIONS

As it stands at the present time, researchers in this area of taste research are concentrating their efforts in investigating the relationship between zinc metabolism and gustatory sensitivity. Henkin and his colleagues have provided a vast amount of valuable background information but still many questions remain to be answered. These questions include:-

(1) Does zinc deficiency as defined by a decrease in serum or plasma zinc concentration produce hypogeusia in all or most cases?

(2) Will all patients with decreased serum or plasma zinc concentrations who exhibit symptoms of hypogeusia benefit from zinc medication? i.e. will hypogeusia be improved in all or most cases with the administration of zinc ion?

(3) Is there any correlation between the degree or extent of hypogeusia and serum or plasma zinc concentration?

(4) It has been shown that the wound healing rate improves with the administration of zinc compounds either orally or topically. If oral administration of zinc ion can be shown to correct hypogeusia, will topical application of zinc ion, i.e. as a mouth rinse, have the same effect?

In the chapters to follow, attempts will be made to answer these questions by experimentation and relevant discussions.

CHAPTER V

EXPERIMENTS ON ZINC METABOLISM AND TASTE SENSITIVITY

In the previous chapters, we have summed up the results of relevant studies into the possible relationship between zinc metabolism and gustatory sensitivity. There are still many gaps in the knowledge of this area which need to be filled before full understanding of the mechanisms involved in this relationship could be attained. Schechter et al. (1972), in reporting the success of their treatment of idiopathic hypogeusia with zinc sulphate medication concluded that:

> "At present it is not possible to predict which patients will improve on zinc therapy. No correlation can yet be made between etiology of the syndrome, duration of symptoms, initial serum and urinary metal concentrations and the effectiveness of zinc."

Besides the above points, many other questions are still to be answered. So far, there has been no report in the literature which traces the changes in zinc metabolism during treatment with zinc medication. The closest available information in this area is the study with isotopic zinc which traces the path taken by the zinc medication in the body after a single administration of the isotopic zinc. Information concerning the changes in zinc metabolism, e.g. the changes in zinc content of the body as represented by changes in the amount of zinc in serum or plasma during the treatment period, are needed before any firm statement connecting changes in zinc metabolism and gustatory functioning can be made.

As pointed out earlier in this thesis, the study of taste alterations and zinc metabolism so far has been conducted in patients with rare diseases. Even in the cases of idiopathic

hypogeusia, the alterations in taste of the patients are severe and not commonly found in general clinical practice. No study has yet been reported of the possible application of zinc therapy among patients with taste alterations of a less severe magnitude.

It is well known that patients suffering from various diseases often complain of a lack of appetite and that "everything tastes the same" or that "nothing has much taste to it any more". These apparent changes in taste sensitivity are not so severe as to be distressing to the patients as in the cases of idiopathic hypogeusia but is sufficient to cause discomfort to the patient. It is the objective of this research to determine the effectiveness of zinc treatment in this situation.

The general plan of this research, therefore, is objectively to study the changes in taste sensitivity in a particular group of patients who experience subjective reduction in taste sensitivity and to observe the possible changes in taste sensitivity during treatment with zinc medication.

Hospitalized chronic alcoholic patients have been chosen as subjects of this study because of two reasons. Firstly, the majority of chronic alcoholic patients present themselves at admission to the hospital in an undernourished state with deficiencies in several dietary elements. Sullivan and Lankford (1962,1965) reported decreased serum zinc concentration, excess urinary zinc excretion and increased renal clearance of zinc among chronic alcoholics both with and without abnormal liver functioning. Therefore, there is a probability that hospitalized chronic alcoholics are deficient in their total body content of zinc at admission to the hospital.

Secondly, as Smith (1972) pointed out, alcoholic patients frequently complain of reduction in taste sensitivity. He reported

a significant increase in objective taste thresholds to quinine among alcoholic subjects in his study when compared with matched control healthy subjects. This result was viewed as supporting the assertion that prolonged alcoholic consumption impairs taste sensations. These two reasons seem to indicate that some alcoholic patients may benefit from zinc medication both for its nutritional value and the possibility of the treatment improving the patients' taste sensitivity. The above reason together with the comparatively large number of hospitalized alcoholic patients in the local hospitals makes them appropriate as the subjects of this study.

This chapter contains an account of the experiments conducted to study the changes in taste sensitivity among hospitalized alcoholic patients and the results of treatment with zinc sulphate medication.

I. CHANGES IN SERUM ZINC CONCENTRATION DURING TREATMENT WITH ZINC SULPHATE MEDICATION

Before any study can be made of the possible effect of zinc metabolism upon taste sensitivity employing zinc medication, a major question must be answered. How much medication to give and what effect does this dosage of the zinc medication have on the zinc supply in the body?

In the past, zinc sulphate in the form of capsules has been chosen as the best zinc compound to be used when zinc treatment is required. This is because of its low toxicity and high deionizing rate. Several dosages of zinc sulphate have been administered in different situations. Henkin and his colleagues, in treating idiopathic hypogeusia administered 100 mg zinc ion (440 mg zinc sulphate) daily, divided in four equal doses taken four times a

day. After three months of this "loading dose", they retained the patients on "maintenance" therapy of 25 mg of zinc ion daily (Henkin, Personal Correspondence, 1973). Pories (1967) used zinc sulphate at the dosage of 220 mg three times daily (a total of 660 mg per day) in the successful treatment of patients with pilonidal sinus wounds.

The daily dosage of 660 mg zinc sulphate, yielding 150 mg zinc ion daily has been widely used in treating patients with chronic ulceration. In various published documents in the literature which deals with toxicity, this dosage of zinc sulphate does not produce any toxic effects in the patients to whom the medication has been administered (Pories 1967). Also, of all the dosages used in different studies, this dosage of zinc sulphate seems to produce the desired effect in the shortest period of time. Therefore, the administration of 220 mg of zinc sulphate three times daily was considered a possible dosage to be used in this series of experiments.

In order to study the effect of the administration of 150 mg zinc ion supplementation on the supply of zinc in the human body, a brief experiment has been conducted. The aim of this experiment is to study the changes in the amount of zinc in the body as indicated by the changes in zinc serum concentration during the period of zinc therapy.

(a) Subjects. Three patients who were admitted to the local hospital for the treatment of chronic leg ulcers took part in this experiment. They were two female patients aged 46 and 63 years and a 50 year old male patient. These patients underwent treatment with zinc sulphate medication while being confined to bed after admission as part of their treatment programme along similar lines to the treatments reported by Pories (1967) and others.

(b) Method. Upon admission, the patients had their blood samples taken and analysed for serum zinc content. When the results of the analyses were known, usually a few days after admission, and the results indicated that the patients' serum zinc concentration was lower than 110 µg/100 ml, the patients were given zinc sulphate medication at the dosage of 660 mg zinc sulphate daily. The medication was in the form of capsules each containing 220 mg of zinc sulphate powder, one capsule is taken three times daily after meals. The period of treatment for each patient was determined by the surgeon concerned.

During the treatment period, blood samples were taken from each patient at two-day intervals or as frequently as practicable. The samples were taken and analysed by the staff of the Pathology Department at the hospital. The blood samples were analysed for zinc content of the serum by the electron spectrophotometry method. Great care was taken to avoid contamination of the samples and haemolysis.

During treatment with zinc sulphate medication, the patients were not given any other medication and were retained under normal hospital diet.

(c) Results and Discussion. The changes in serum zinc concentration of the three patients during treatment with zinc sulphate medication are shown in fig. V.1.

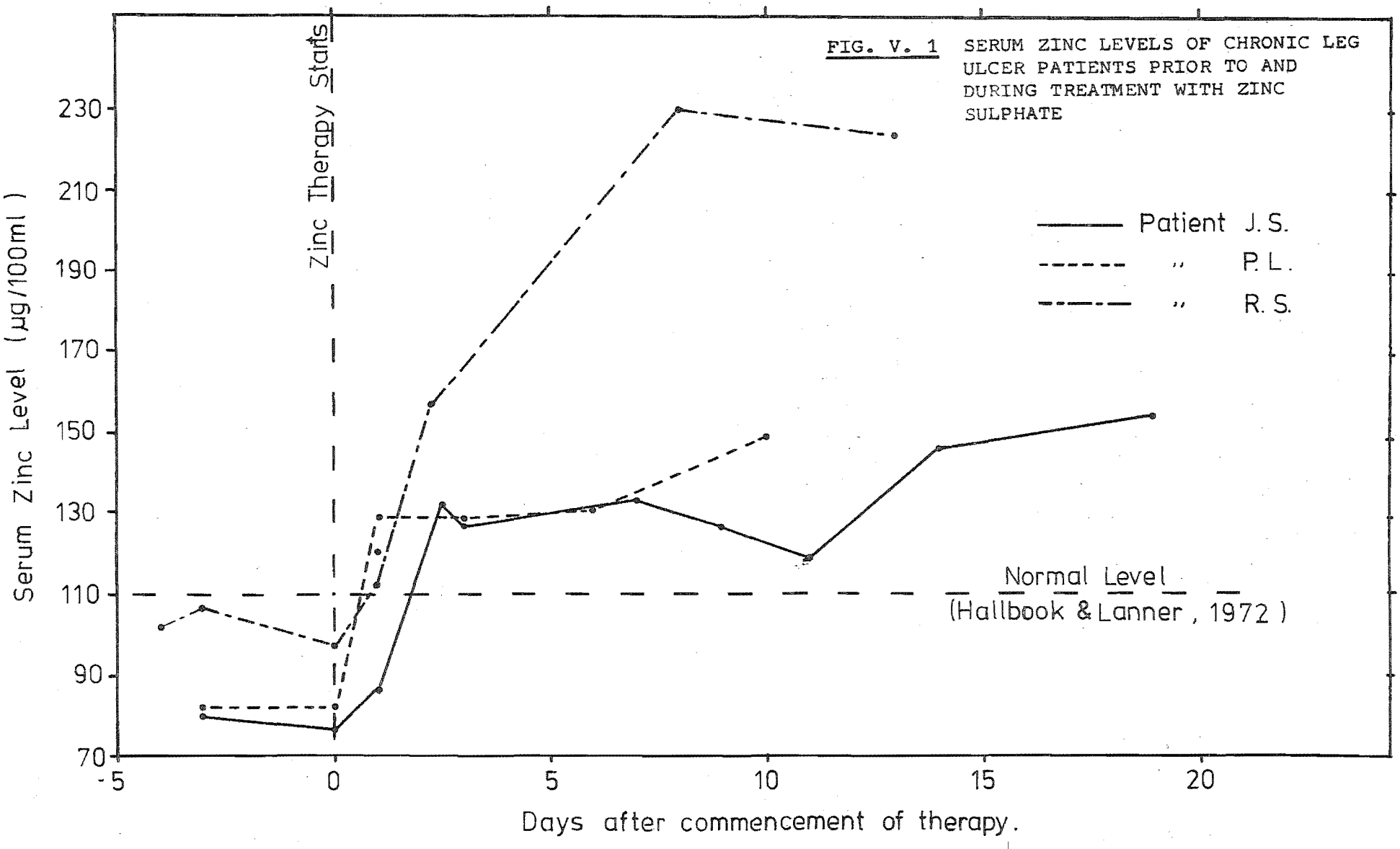


FIG. V. 1 SERUM ZINC LEVELS OF CHRONIC LEG ULCER PATIENTS PRIOR TO AND DURING TREATMENT WITH ZINC SULPHATE

Right through this thesis, a serum zinc concentration of 110 microgrammes per 100 millilitres is regarded as the normal level following the report of Hallbook and Lanner (1972). It is also to be noted here that in the experience of the pathologist who performed all the analyses of blood samples in this thesis, serum zinc and plasma zinc content are identical in value, hence the two terms will be used interchangeably.

It can be seen from fig. V.1., that all three patients had serum zinc concentration below 110 µg/ 100 ml upon admission. The administration of 660 mg daily of zinc sulphate raised the serum zinc concentration levels above the normal value within the first two days of the treatment. Even though there were wide individual differences among the three patients in the rate of uptake of zinc in the serum, it still can be seen that the serum zinc concentration levels remained higher than the normal level right through the treatment period.

Therefore, it is possible to conclude from the available data, that zinc sulphate medication at the dosage of 660 mg daily was successful in raising the serum zinc concentration to and retaining it above the normal level in these patients. With the possible exception of patients suffering from malabsorption, preventing the normal absorption of zinc into the blood, similar rises in serum zinc levels can be expected in other patients undergoing treatment with this dosage of zinc sulphate.

The limited number of subjects used in this study prevents further investigation into the relationship between the rate of zinc uptake by the serum and the pretreatment serum zinc level. This information may be useful in predicting the changes in serum zinc concentration and allowing more definite conclusions to be drawn concerning the administration of zinc sulphate medication

and its effects on serum zinc concentration. It was not possible to include more subjects in this study due to the scarcity of patients with chronic leg ulcers being admitted to the local hospitals.

The rise in serum zinc concentration is regarded as reflecting the greater availability of zinc in the body of the patient. The success in raising serum zinc concentration above normal level and retaining it at this high level is regarded as indicating the success in correcting the previously zinc deficient state of the patient as discussed in Chapter II of this thesis.

It can be concluded that the daily administration of 660 mg of zinc sulphate is successful in raising serum zinc concentration to normal level within the first two days of treatment and that the serum zinc level is maintained above normal level as long as medication is continued.

II. TASTE SENSITIVITY IN ALCOHOLIC PATIENTS

As mentioned earlier, many alcoholic patients who are admitted into a hospital for the treatment of alcoholism complain of decreased taste sensitivity. To the questions relating to their eating habits, these patients report general lack of appetite due to reduction in the ability to differentiate between different tastes. In their own words, "everything tastes the same to me". Objectively, Smith (1972) reported a significantly higher threshold to quinine among alcoholics when compared with normal control subjects.

Physically, many alcoholics exhibit symptoms of malnutrition due to prolonged alcoholic intake and lack of well balanced food intake, prior to the time of their admissions to the hospital. Sullivan and Lankford (1965) in their study of 125 patients admitted

into a hospital with acute or chronic alcoholism, reported high incidences of decreased serum zinc concentration, excess urinary zinc excretion and increased renal clearance of zinc. These symptoms appear in both patients with and without signs and symptoms of hepatic dysfunctions. They further reported that in the majority of these patients, the alterations in zinc metabolism is transient and zinc metabolism returns to normal within one to two weeks following abstention from alcohol and an adequate diet. In patients with post-alcoholic cirrhosis, however, these alterations in zinc metabolism may persist for long periods and do not improve with adequate diet alone (Sullivan 1962).

With the above two sets of information, one may speculate that if the hypothesis of Henkin and his associates, concerning the relationship between zinc metabolism and taste sensitivity is applicable to all types of patients, the apparent decrease in taste sensitivity experienced by the majority of chronic alcoholics is due to the alterations in zinc metabolism. Among these alcoholic patients who do not show any sign of liver dysfunction and whose zinc metabolism is expected to return to normal within the first two weeks of hospitalization without zinc supplementation, taste sensitivity may be expected to return to normal within the first two weeks.

(1) Experiment Alcoholic Taste I

To test the above hypothesis, an experiment was conducted involving the patients admitted to the Queen Mary Hospital, Hanmer Springs. The aim of this experiment was to study objectively the taste sensitivity of alcoholic patients upon admission into the hospital and to study the changes in taste sensitivity which may occur during the first two weeks of admission.

(a) Subjects. The subjects were 12 male patients aged

between 21 and 60 years old with a mean age of 49.167 years. These patients were admitted to the hospital for the treatment of chronic alcoholism. Their participation in this study was voluntary and each patient had the brief objectives of the experiment explained to them. Each patient underwent a routine physical examination which revealed no obvious sign of liver dysfunction. None of these patients underwent treatment by medication for any other medical complaints. All of the patients smoked cigarettes habitually and their smoking habits did not change during the study period. Every one of the twelve patients subjectively reported decreased taste sensitivity prior to admission to the hospital.

(b) Method. Each patient was assessed for their taste sensitivity during three sessions at weekly intervals starting from the day after their admission. This schedule provided information concerning taste sensitivity of the patients at admission and during the first two weeks of their hospitalisation.

In the first session, each patient had the objectives of the assessment explained to them in order to gain their cooperation right through the three sessions. The patients' involvement was voluntary and patients were free to decline to participate in the study.

In each session, each patient was asked to taste a series of solutions of glucose, sodium chloride, citric acid and quinine sulphate in deionized water of different concentrations together with water blanks. The concentrations of the solutions used were as follows.

TABLE V.1. CONCENTRATION OF VARIOUS SOLUTIONS USED (gm/litre)

SOLUTIONS	1	2	3	4	5
Glucose (A)	6.25	12.50	25.00	50.00	100.00
Sodium Chloride (B)	0.46875	0.9375	1.875	3.750	7.50
Citric Acid (C)	0.01875	0.0375	0.075	0.150	0.300
Quinine Sulphate (D)	0.0025	0.005	0.01	0.02	0.040

These solutions were selected from a number of solutions used in preliminary pilot studies, conducted to determine suitable concentrations of chemicals to be used with alcoholic subjects. The solutions were prepared in the Gustatory Psychophysics Laboratory at the University of Canterbury. The deionized water used to dissolve the chemicals was deionized to a resistance of not less that 2 mega ohms per centimetre (equivalent to triple distilled).

In one session, each patient was asked to taste four series of six stimuli, twenty solutions and four water blanks. The stimuli in each set were randomized in quality but in ascending order of concentration. An example of such arrangement is as follows:

A1	B2	∅	C3	A4	D5
C1	D2	A3	B4	∅	C5
B1	A2	D3	∅	C4	A5
D1	∅	C2	B3	D4	B5

where ∅ denotes water blanks.

Each stimulus was 10 ml of either a solution or deionized water, contained in a 50 ml pyrex beaker from which the subject

took the content completely into his mouth. The solutions were kept at room temperature. The time sequence of tasting was not strictly controlled as the individual subject was allowed to adopt his own rate of responding. There was roughly a one-minute interval between each solution tasted.

The instructions given to the subjects were as follows:

"This study consists of a number of solutions for you to taste. The whole of the solution in each beaker is to be taken into your mouth and rolled around your tongue while you decide if it is water, sweet, salty, bitter or sour. It may have some taste that you cannot give a name to which we will just describe as 'Taste'.

When you decide what the solution tastes like, I want you to indicate to me on the card (show card) in front of you. Then you can spit it out or swallow it as you will.

Before we start, rinse your mouth. After each series you are to rinse out again, I will indicate when."

On a piece of white cardboard laid in front of the subject, six words were written in clear block letters. These six words were WATER, TASTE, SWEET, SALT, SOUR and BITTER. The subject was asked to point to one of these words to convey their responses to the stimuli.

All testing was done at roughly the same time in the morning. The subjects were asked to refrain from eating or smoking a half hour prior to testing. All testing took place in the office of the hospital pharmacist who also took part as an experimenter in some sessions.

(c) Results and Discussion. The recorded results were analysed, employing the MANOVA computer program supplied by the Psychometric Laboratory, University of North Carolina.

The mean concentrations of correctly identified stimulus solutions in each of three sessions were compared for each stimulus quality. The unit used is what Henkin calls "bottle unit" which is the concentration step at which the subject reports correct

identification of taste quality (Table V.2).

TABLE V.2. MEANS AND STANDARD DEVIATIONS IN CONCENTRATION (BOTTLE) UNITS OF CORRECTLY IDENTIFIED STIMULUS SOLUTIONS WITH MEAN AGE OF SUBJECTS IN EXPERIMENT ALCOHOLIC TASTE I

SESSION		SWEET	SALT	SOUR	BITTER	AGE (Years)
FIRST	$\bar{X}$	4.250	3.917	3.917	3.167	49.167
(1 day after Admission)	S.D	1.288	1.782	2.151	1.850	12.561
SECOND	$\bar{X}$	3.833	4.500	3.833	3.000	49.167
(8 days after Admission)	S.D	0.937	1.243	1.687	1.859	12.561
THIRD	$\bar{X}$	4.273	3.455	3.636	2.909	49.167
(15 days after Admission)	S.D	1.104	0.688	1.567	1.758	12.561

To determine whether significant changes in taste perception took place among these alcoholic patients in a two week period, two MANOVA analyses had been performed.

In the first analysis, the age of the patients was used as a covariate, while the concentrations of correctly identified stimuli were the criteria variables. This analysis showed that age as a covariate did not have any significant effect on the criteria variables ($p < .475$).

In the second analysis, no covariate was considered. Some of the tables provided in the MANOVA print out are as follows:

TABLE V.3. WITHIN CELL CORRELATIONS, WITH STANDARD DEVIATIONS IN THE DIAGONAL CELLS ONLY

VARIABLE	SWEET	SALT	SOUR	BITTER
SWEET	1.119			
SALT	0.186	1.330		
SOUR	0.229	0.292	1.830	
BITTER	0.501	0.201	0.569	1.825

TABLE V.4. UNIVARIATE F VALUES, SIGNIFICANT LEVELS AND STANDARDIZED DISCRIMINANT FUNCTION COEFFICIENT (SDF) OF EACH CRITERIA VARIABLES.

VARIABLE	F (2,32)	P LESS THAN	SDF
SWEET	0.576	0.568	0.702
SALT	1.787	0.184	-0.908
SOUR	0.070	0.932	0.193
BITTER	0.059	0.942	-0.301

TABLE V.5. MULTIVARIATE F VALUES FOR TREATMENT EFFECT AND CANONICAL CORRELATION (R)

TEST OF ROOTS	F	DF	P LESS THAN	R
1 THROUGH 2	0.650	8,58	0.732	0.391
2 THROUGH 2	0.055	3,29	0.982	0.075

From the above tables, it is clear that there was no significant change in perception of taste of the four types of solutions used, during the first two weeks of hospitalisation.

All twelve patients gave subjective reports of decreased taste sensitivity for all taste qualities. Even though this experiment was designed to study changes in taste perception over time, there was evidence in support of the suggestion that alcoholic patients showed objective decrease in taste sensitivity. The concentrations at which the subjects correctly identified taste qualities were higher than those reported by past researchers (Amerine, Pangborn and Rossler, 1965) in all taste qualities. These decreased taste sensitivities did not improve during the two-week period in which this study took place. However, all the subjects reported subjective improvement in taste functioning together with increase in appetite and a general sense of well being characteristic of alcoholic patients after their "drying out" period.

Lack of facilities prevented determinations of zinc serum or plasma concentrations at admission, during and at the end of the study period. This is the most important weakness of this study. As it was, the best conclusion which could be drawn from this study is that there was no change or improvement in taste sensitivity among these subjects during the first two weeks of their hospitalisation, during which time zinc metabolism was assumed to have returned to normal levels according to Sullivan (1962).

The method used in determining taste sensitivity in this study may be criticised as inadequate, as only one sample of each concentration in a stimulus series was presented in any one session. This is due to the shortness of attention and concentration span in chronic alcoholics. In pilot studies where a total of 50 samples were presented, in two trials of 25 samples each, the second trial was responded to randomly, showing that the subjects had lost the attention required to make objective judgements. In view that this study was designed to investigate changes in sensitivity over a period of time and not to determine threshold per se, this weakness in method may not be so serious, as in the case of threshold determination.

The selection of stimulus concentrations used in this and subsequent studies was done by pilot studies. It was necessary to find a series of solutions which would cover the range of sensitivity in the majority of alcoholic patients, while minimizing the number of solutions used in each series and selecting the series of concentrations with minimum difference between each successive concentration which would reveal any change in sensitivity which might occur over time. The series of solutions used in this and subsequent studies (Table V.1) appeared to be best

suited to the above requirements.

(2) Experiment Alcoholic Taste II

It was felt during the previous experiment, that the two-week period of hospitalisation during which the taste tests were conducted, may not be long enough to allow any change in taste sensitivity to take place. Another experiment was carried out which extended the period of study to three weeks. This experiment was intended to study the possible changes in taste sensitivity among hospitalised alcoholic patients during the first three weeks of their admission into the hospital.

(a) Subjects. The subjects were 9 male and 1 female patients who were admitted as in-patients to the Alcoholic Unit at Sunnyside Hospital, Christchurch, for treatment of alcoholism. The mean age of these patients was 39.8 years (range 29 to 49 years).

In comparison to the group studied in the previous experiment, this group of patients did not show any major difference in medical or psychiatric history. All of the patients in this group smoked habitually and wore some forms of denture. There were no symptoms or signs of hepatic dysfunction. None of the patients involved in this study was taking medication other than multi-vitamin and Hemineurin during the first four days of admission and Chlorpromazine 100 mg nocte, which was the normal medication schedule for alcoholic patients admitted to this hospital.

All of the ten subjects reported subjective decrease in taste sensitivity prior to admission to the hospital.

(b) Method. In this experiment, each subject had his taste sensitivity to glucose, sodium chloride, citric acid and quinine sulphate assessed in four sessions at weekly intervals

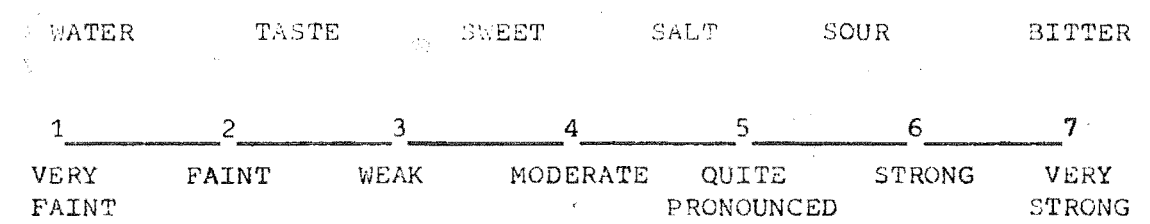
starting from the day after admission.

The concentration of solutions used, the order and manner in which the samples were presented to the subjects were the same as those described in Experiment Alcoholic Taste I.

The manner of responding to the stimuli was, however, different. In this experiment, at the beginning of each session, the subject was asked to taste a series of four solutions as follows:

Glucose	100.0 gm/lt
Sodium Chloride	7.5 gm/lt
Citric Acid	0.3 gm/lt
Quinine Sulphate	0.04 gm/lt

The subject was told that these solutions represented the four basic tastes and were of the strongest concentrations to be used in the stimulus series. They would be rated as very strong, on a scale placed in front of the subject. The subject was then asked to use this scale in making his responses to each of the sample solutions presented to him. To each stimulus, the subject was asked to judge whether it was water or taste (detection of a taste but unidentifiable) or sweet, salt, sour or bitter. If the taste of the stimulus was identifiable by the subject, he was asked to rate the intensity of the taste according to the following seven point scale:



The aim of the experiment was explained to the subjects and their cooperation sought in the same manner as in the previous

experiment. All the assessments were carried out between 1.30 and 2.30 p.m. in the clinic room of the unit. The subjects were asked to refrain from eating and smoking, a half-hour prior to each session.

A brief medical history of each patient and information concerning his age, smoking habits, wearing of dentures and medication taken was compiled on a questionnaire form. (Appendix I)

(c) Result and Discussion. The results obtained from these ten subjects over four sessions covering the first three weeks of admission were considered in two separate sets of analyses.

Firstly, to determine whether any change in recognition of the appropriate taste qualities took place during this period, the results in terms of "bottle units" were analysed by MANOVA in the same manner as that reported in the previous experiment. The means and standard deviations in concentration (bottle) units of correctly identified stimulus solutions from each of the four sessions were compared and tabulated as follows:

TABLE V.6 MEANS AND STANDARD DEVIATIONS IN CONCENTRATION (BOTTLE) UNITS OF CORRECTLY IDENTIFIED STIMULUS CONCENTRATIONS WITH MEAN AGE OF SUBJECTS IN EXPERIMENT ALCOHOLIC TASTE II

SESSION		SWEET	SALT	SOUR	BITTER	AGE (years)
FIRST	$\bar{X}$	4.000	3.900	4.800	4.600	39.800
	S.D.	1.054	1.101	1.229	1.506	6.713
SECOND	$\bar{X}$	3.600	4.300	4.400	4.500	39.800
	S.D.	0.843	1.252	0.699	1.080	6.713
THIRD	$\bar{X}$	3.500	4.300	3.800	3.800	39.800
	S.D.	1.080	1.252	1.135	1.476	6.713
FOURTH	$\bar{X}$	3.400	3.700	3.500	3.500	39.800
	S.D.	1.075	1.337	1.354	1.841	6.713

The first MANOVA performed on this set of data employed the correct identification of sweet, salt, sour and bitter as criteria variables and the age of the subjects as covariate. However, age was found to be non-significant in its effect on the sensitivity to the solutions used ($P < 0.454$).

The second MANOVA was performed without covariate. The computer print out contained similar tables to the ones reproduced in the previous experiment. The results revealed no significant changes in sensitivity over the four sessions, as shown in Table V.7.

TABLE V.7. MULTIVARIATE F VALUES FOR TREATMENT EFFECT AND CANONICAL CORRELATION (R)

TEST OF ROOTS	F	DF	P LESS THAN	R
1 THROUGH 2	0.895	12,87	0.556	0.440
2 THROUGH 3	0.520	6,67	0.791	0.283
3 THROUGH 3	0.125	2,34	0.883	0.085

The univariate F tests also did not show any significant changes in sensitivity to each of the four taste qualities investigated over the four sessions.

Therefore, the MANOVA analysis seemed to indicate that there was no change in recognition of the four taste qualities investigated over the four testing sessions carried out during the first three weeks of hospitalisation.

In the other series of analyses on the data from this experiment, the changes in the rating of intensity for each solution in each chemical series over the four-week period were investigated. Disregarding the quality of the taste identified, the intensity of each solution perceived by the subject, as indicated by him on the provided scale, was coded into number form according to the

following system.

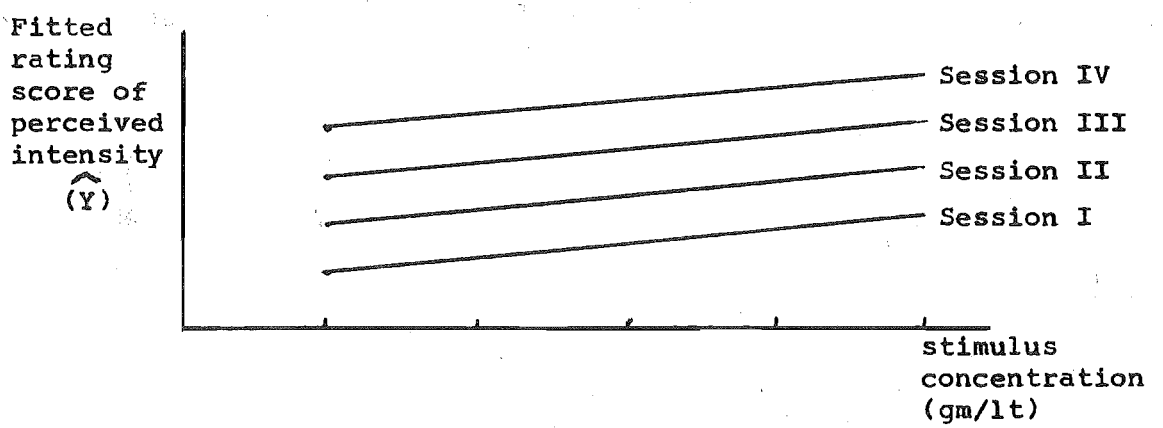
water	=	1	moderate	=	6
taste	=	2	quite pronounced	=	7
very faint	=	3	strong	=	8
faint	=	4	very strong	=	9
weak	=	5			

This coding system extended the scale on which the subjects rated their responses to a nine point scale.

Linear regression analysis was performed on the rating scores which the ten subjects assigned to a particular solution in each session. The solutions were grouped together according to their taste quality in ascending order of concentration in this analysis. The regression resulted in four straight lines on a plot representing the four sessions for each taste quality.

If the hypothesis that taste sensitivity of alcoholic patients improves with time during the first three weeks of their hospitalisation is correct, one would expect a plot of regression lines of the following type for each of the taste qualities.

REGRESSION LINES OF IDEAL SITUATION WHERE TASTE SENSITIVITY IMPROVES OVER FOUR TESTING SESSIONS



That is, we would expect the intercept values to increase over the four sessions, while the value of the slope remains fairly constant over the sessions.

The results of regression analysis of the data from this experiment were tabulated in Table V.8.

TABLE V.8. THE INTERCEPT, SLOPE AND F.RATIO OF EACH REGRESSION LINE REPRESENTING THE RATING SCORES OF PERCEIVED INTENSITY OF A QUALITY IN EACH SESSION

SOLUTION	SESSION	INTERCEPT	SLOPE	F. RATIO
GLUCOSE	I	1.600	0.652	69.031
	II	2.375	0.666	79.828
	III	3.350	0.571	38.295
	IV	4.208	0.423	42.128
SODIUM CHLORIDE	I	2.942	0.381	18.536
	II	3.020	0.381	26.287
	III	5.158	0.183	6.314
	IV	4.195	0.329	23.171
CITRIC ACID	I	2.596	0.343	15.519
	II	3.987	0.288	15.020
	III	4.233	0.233	7.778
	IV	4.770	0.282	24.710
QUININE SULPHATE	I	3.112	0.375	15.829
	II	4.008	0.345	14.768
	III	5.558	0.232	15.425
	IV	5.942	0.209	10.362

The results of this regression analysis seemed to indicate that there were slight changes in ratings of perceived intensity of each stimulus recorded by these subjects in the four sessions studied. The general trend seemed to be that a change towards increase in intensity perceived occurred in the second session with smaller changes in the third and fourth sessions. These slight changes in perceived intensity of solutions over the four sessions were not sufficient to support the hypothesis of significant

changes in taste sensitivity among alcoholic patients.

Considering both sets of analyses together, it seems that slight changes in taste sensitivity took place during the first three weeks of hospitalisation but is not sufficient to create significant differences in taste sensitivity in all taste qualities over the four sessions. Overall, there seemed to be little support to the hypothesis that taste sensitivity of alcoholics improves significantly during the first weeks of their hospitalisation. Any relation to zinc status of these patients could not be made because no zinc analysis was carried out at this stage.

Subjectively, all of the ten subjects reported decreased taste sensitivity prior to their admission and reported great improvements in their taste sensitivity during the three week period of study.

The reason for the introduction of the rating scale into this experiment was that it was felt that the method used in the previous experiment was inadequate. While acknowledging that the measurement of thresholds, both recognition and detection are cumbersome and inappropriate for the purposes of this thesis, there was a need to investigate the changes over time in both recognition and detection aspects of taste functioning. By dealing with the correct identification of taste qualities investigated, the recognition functioning was considered. It was thought that, by using a rating scale, it might be possible to obtain more information concerning the detection of taste. There might be an increase in perceived intensity of a solution while the subject was not able to identify the quality of the solution correctly, for example, while a subject named a solution of sodium chloride, sweet in three sessions, there may have been differences

in the degree of sweetness experienced by him from this solution over this time period.

The rating scale was also used in an attempt to deal with the taste confusion phenomenon. Besides the well documented sour-bitter confusion experienced by a large portion of the population (Gregson and Baker 1973), it was noticed in the previous experiment and confirmed in this experiment that alcoholic subjects showed a tendency to confuse salt and sour at certain concentrations. It was common (75% of 22 patients in both experiments) for the subjects to respond to 9.375% solution of sodium chloride as being sour.

By giving each subject the four strongest solutions used in this experiment as examples at the beginning of each session and indicating to the subject, the appropriate taste quality of each solution, it was hoped that the confusion arising from the differences in nomenclature would be eliminated. The confused taste quality was then regarded as false recognition as far as preparing the data for MANOVA analyses was concerned. By considering the rating scores without concerning with the quality of taste, any change in perception even though confused in recognition, could still be investigated.

To conclude, from the results of this experiment, without referring to zinc status of the patients, there were no significant changes in taste sensitivity of the ten subjects during the first three weeks of hospitalisation.

III. EXPERIMENT ZINC SULPHATE TREATMENT AND TASTE

The data from the previous two experiments indicated that the chronic alcoholic patients who took part in the experiments, showed both subjective and objective decrease in taste sensitivity

in the four basic taste qualities investigated. Their taste sensitivity did not change significantly during the first three weeks of their hospitalisation.

The next step in this series of studies is to investigate the possible relationship between taste sensitivity and zinc metabolism. There are two questions that need to be answered at this stage.

(a) Do alcoholic patients who experience decreased taste sensitivity both subjectively and objectively at admission to the hospital have lower zinc serum or plasma levels than normal?

(b) Does the administration of zinc sulphate medication which raises the amount of zinc in the body, have any significant effect on taste sensitivity?

If Henkin's hypotheses are applicable in this case we would expect the alcoholic patients with decreased taste acuity to have lower than normal zinc serum and plasma levels. When these zinc levels have been raised up to normal levels by the administration of zinc sulphate medication, we would expect significant changes in taste sensitivity among these patients who received the zinc treatment.

To test these hypotheses, an experiment was conducted among alcoholic patients at the Alcoholic Unit, Sunnyside Hospital, Christchurch.

(a) Subjects. The patients who were admitted to the Alcoholic Unit for treatment of Alcoholism during the six-month period, March - August 1975 took part in this experiment. Ninety five patients gave blood samples to be analysed for plasma zinc content at admission.

Out of these ninety five patients, forty patients who complained of subjective decrease in taste sensitivity took part in

the main study. They were divided into two groups to be treated with either zinc sulphate medication or placebos for a period of three weeks. During the three week period, many patients were discharged either with or without medical approval. The final numbers of subjects in each group were eighteen in the zinc sulphate medication group and seven in the placebo group.

These twenty five patients who were all males, complained of subjective decrease in taste sensitivity prior to admission. They did not show definite signs or symptoms of hepatic dysfunctions. They all smoked habitually and wore dentures to some extent. They were not under medication for any complaints other than alcoholism. They were all given multivitamin and Hemineurin during the first week of hospitalisation.

The ages and zinc plasma levels at admission distribution of the two groups were as follows:

Experimental Group (Zinc sulphate medication), 17 subjects
Age 40.889 ± 8.970 years
Zinc plasma at admission 93.944 ± 12.567 µg/100 ml

Control Group (Placebo), 8 subjects
Age 40.429 ± 11.759 years
Zinc plasma at admission 92.714 ± 11.968 µg/100 ml

(b) Method. All the ninety five patients mentioned had their blood samples taken on the morning after their admission to the Alcoholic Unit or as soon as practicable. These samples were analysed for zinc plasma concentration by the electron spectrophotometric method by the staff of the Pathology Department, The Princess Margaret Hospital.

Within the first two days of admission, each of these patients was seen in a brief interview with the experimenter who inquired about the subjective taste functioning of the patient, his or her eating habits and brief medical history. Those patients who reported subjective loss or decrease in taste sensitivity were

asked to take part in the main experiment. Originally, there were forty patients under this category. Each patient was told that he would be given an objective test of his taste functioning and if the result was not satisfactory he would be asked to participate in a follow up study of three weeks. It was explained to the patient that taste functioning may be affected by nutritional factors and that we were interested in observing the relationship between some dietary components and taste functioning in alcoholics. The patients were told that during this three-week period of study, they would be given medication which would correct the nutritional deficient state of their bodies. The patients were not told how this medication might affect their taste functioning.

The forty patients then took part in a single blind experiment. They were randomly assigned to one of the two following groups.

The Experimental Group The patients in this group were given a week of placebo medication 3 times daily after meals, followed by 2 weeks of zinc sulphate medication given in clear gelatin capsules at the dosage of 220 mg zinc sulphate, 3 times daily after meals, a total of 660 mg zinc sulphate or 150 mg zinc ion per day.

The Control Group The patients in this group were given three weeks of placebo medication three times daily after meals. The actual placebo medications were made up in gelatin capsules which were indistinguishable from the zinc sulphate medication.

The patients did not know which of these two groups they were assigned to. During the three weeks period, the patients were given normal hospital diet.

Each patient had his blood sample taken for the analysis

of plasma zinc content on the morning after the last dosage of medication had been taken. All blood samples were taken between 8.00 and 8.30 a.m.

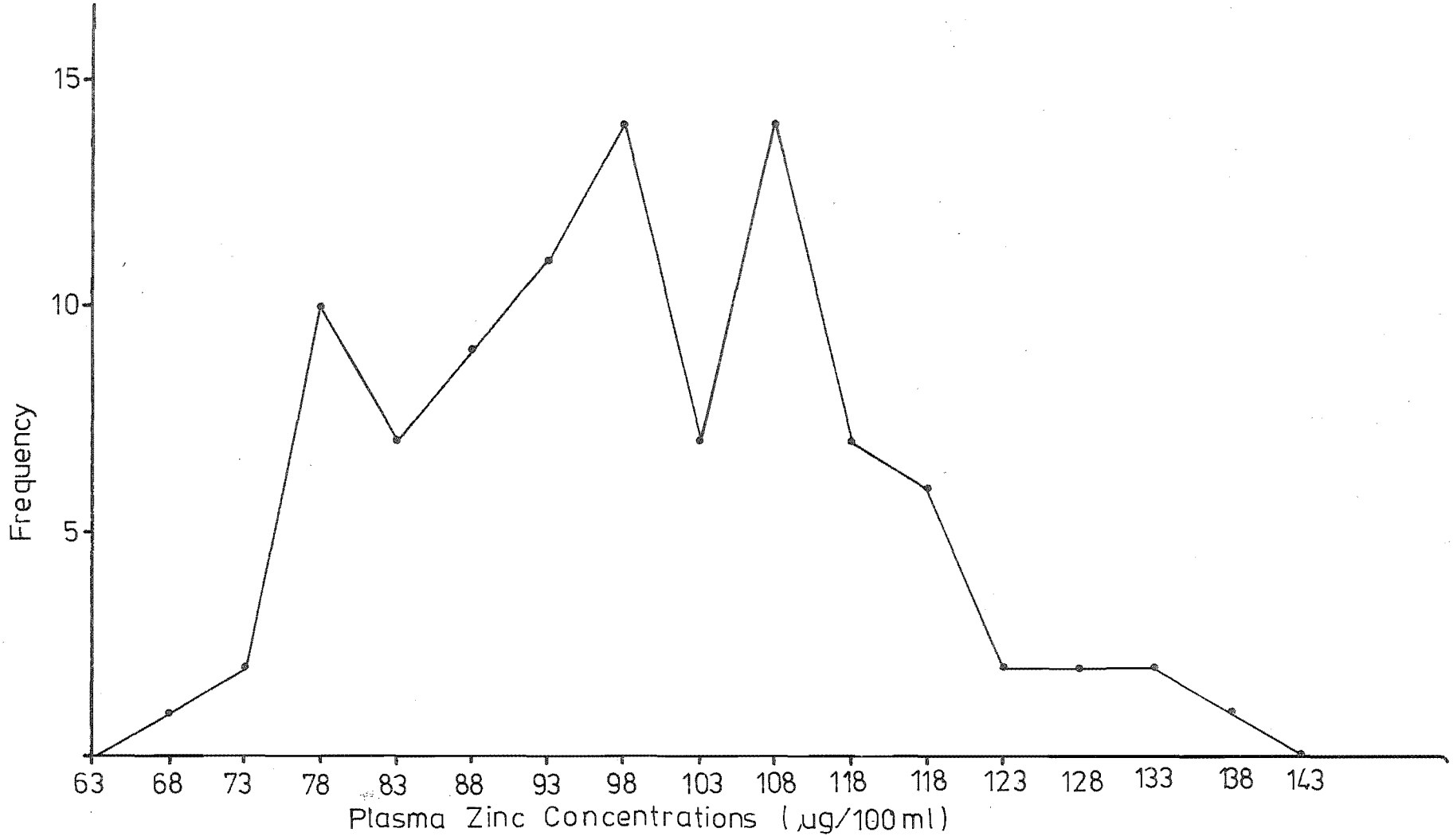
During the three-week period in which the patients underwent treatment, four objective taste assessments were carried out at weekly intervals starting from the day prior to commencement of therapy. All assessments were done between 1.30 and 2.30 p.m. The patients were asked to refrain from drinking and eating, half an hour prior to the assessments.

The solutions used and the method employed in the assessment were the same as described in the previous experiment with the addition of zinc sulphate solution. It was found that zinc sulphate solution produced a sensation of dryness of the mouth in some patients and in varying degree according to concentration (to be discussed in the next chapter). It was of interest to investigate whether there were significant changes in response to zinc sulphate solutions during the treatment with zinc sulphate medication.

The concentration of zinc sulphate solutions used in this experiment were 0.25, 0.5, 1.0, 2.0 and 4.0 gm/lt.

The Taste test used in this experiment therefore, consisted of 5 series of 6 samples (25 solutions and 5 water blanks). The patients were given the four strongest solutions of glucose, sodium chloride, citric acid and quinine sulphate as examples in the manner described previously. The patients were told to report any sensations of dryness in their mouths, which was taken as their recognition of zinc sulphate solutions. The rating scale used in this experiment was:-

FIG. V. 2 DISTRIBUTION OF PLASMA ZINC CONCENTRATION OF ALCOHOLIC PATIENTS AT ADMISSION



TASTE DRY

WATER SWEET SOUR SALT BITTER

1——2——3——4——5——6——7

very faint faint weak moderate quite pronounced strong very strong

(c) Result and Discussion. The distribution of zinc plasma concentration at admission of ninety five alcoholic patients is shown in Fig.V.2. The mean plasma zinc concentration level of this group of patients was found to be 99.2 µg/100 ml with the standard deviation being 14.8172 µg/100 ml.

In the experience of the pathologist who analysed the blood samples for the zinc content, plasma and serum zinc concentration levels are identical. Only twenty of the ninety five patients in this group had plasma zinc concentration levels higher than 110 µg/100 ml, the normal level of serum zinc concentration according to Hallbook and Lanner (1972).

This finding seems to be in agreement with those of Sullivan and Lankford (1962, 1965) discussed earlier.

The patients who took part in the main section of this experiment were those who reported subjective decrease in taste sensitivity. The mean plasma zinc concentration (expressed with standard deviation values) of the subjects in the experimental groups were 93.944 $\pm$ 12.567 µg/100 ml and 92.714 $\pm$ 11.968 µg/100 ml respectively. These values are well below the normal plasma zinc concentration level.

The means and standard deviations in concentration units of the stimulus solutions correctly identified by the subjects in the two groups over four sessions were compared and tabulated as follows.

TABLE V.9. MEANS AND STANDARD DEVIATIONS IN CONCENTRATION (BOTTLE) UNITS OF THE STIMULUS CONCENTRATION CORRECTLY IDENTIFIED BY THE SUBJECTS IN TWO GROUPS OVER FOUR SESSIONS

GROUP	SESSION		SWEET	SALT	SOUR	BITTER	DRY
EXPERIMENTAL $ZnSO_4$ medication	FIRST	$\bar{X}$	3.722	3.778	3.444	3.222	5.111
		S.D.	1.447	1.396	1.294	0.878	1.231
	SECOND	$\bar{X}$	3.333	3.556	3.111	2.667	4.556
		S.D.	0.767	1.042	0.900	1.029	1.653
	THIRD	$\bar{X}$	2.722	3.111	3.000	2.389	2.889
		S.D.	0.958	0.963	1.085	1.092	1.491
	FOURTH	$\bar{X}$	2.500	2.611	2.778	2.333	2.667
		S.D.	0.985	1.195	1.437	0.907	1.138
CONTROL (Placebo)	FIRST	$\bar{X}$	3.857	4.000	3.714	3.857	4.714
		S.D.	1.345	1.732	1.890	1.345	1.890
	SECOND	$\bar{X}$	2.857	3.286	3.857	2.857	4.571
		S.D.	0.378	0.488	1.464	1.069	1.618
	THIRD	$\bar{X}$	3.286	3.286	2.857	2.714	3.857
		S.D.	0.756	1.496	1.345	1.113	1.952
	FOURTH	$\bar{X}$	3.000	2.571	2.857	2.286	3.429
		S.D.	0.816	1.134	2.268	0.951	1.618

A series of MANOVA analyses had been performed on the data using the correct identification of sweet, salt, sour, bitter and dry as criteria variables and age, plasma zinc levels at admission as covariates.

The MANOVA analyses were carried out to test for significance in three factors.

(1) The changes in taste functioning over the four sessions of assessment (S)

(2) The effect of medication i.e. the difference between

the experimental and control groups(M)

(3) The interaction effect between the above two factors (MS)

Only when plasma zinc levels at admission were used as covariate did we have a significant root ($p<.001$) in the test of within cells regression by the Wilks Lambda Criterion. This indicated that plasma zinc level at the time of admission to the hospital had a significant effect on the result of the taste assessment.

The results of the MANOVA analysis is shown in Table V.10.

TABLE V.10. MULTIVARIATE F VALUES FOR TREATMENT EFFECTS AND CANONICAL CORRELATIONS (R)

FACTOR	TEST OF ROOTS	F	DF	P LESS THAN	R
S	1 through 2	5.143	15,240.570	0.001	0.718
	2 through 3	0.487	8,175	0.865	0.169
	3 through 3	0.449	3,88	0.719	0.123
M	1 through 1	0.692	5,87	0.631	0.196
MS	1 through 2	0.573	15,240.570	0.894	0.258
	2 through 3	0.307	8,175	0.963	0.153
	3 through 3	0.119	3,88	0.949	0.064

It is clear from the above table that the only significant factor is the changes in taste functioning over time (S). The univariate F values for all criteria variables for this factor is shown in Table V.11.

TABLE V.11. UNIVARIATE F VALUES FOR FACTOR S

VARIABLE	F(3,91)	MEAN SQUARES	P LESS THAN
Sweet	6.588	5.867	0.001
Salt	4.765	6.917	0.004
Sour	1.423	2.703	0.241
Bitter	5.640	5.663	0.001
Dry	12.479	26.947	0.001

The results from this analysis indicated that there was no significant difference in the responses to the taste stimuli between the subjects in the experimental group and those in the control group. There were significant differences in responses to glucose, sodium chloride, quinine sulphate and zinc sulphate over the four assessment sessions, but not in responses to citric acid. Looking at Table V.9, it can be concluded that there were significant improvements in the recognition of the taste solutions but these improvements were not due to the medication given.

The plasma zinc analyses at the end of the treatment for both subjects from the zinc sulphate medication and placebo groups showed that, in the zinc sulphate medication group, the plasma zinc concentration levels rose to levels well above 110 µg/100 ml in all subjects which indicated that zinc supply in the subjects' bodies had been increased over the three week period. In the placebo group, on the other hand, the zinc plasma level did not increase more than 5 µg/100 ml in any subject which suggested that there was no significant change in zinc supply and availability of the subjects during the three week period. This also indicated that Sullivan and Lankford's (1962) findings concerning the return

to normal of zinc blood levels among alcoholics did not hold true in these subjects.

Regression analyses of the rating scores obtained in this experiment were also carried out in the same manner as described in the previous experiment. The results showed similar trends to those tabulated in Table V.8. The results suggested changes in perception of intensity in all qualities in both control and experimental groups over the four sessions.

The overall results indicated that, except for citric acid, there were significant changes in taste perception in all the twenty five subjects regardless of the medication which they were given. These changes took place over the three weeks of hospitalisation in which the experiment took place.

The finding that plasma zinc concentration levels of the subjects at the time of their admission to the hospital was a significant covariate with their responses to the taste stimuli, would at first seem to suggest that zinc played a significant part in taste perception. However, we also found that there were significant changes in responses to taste stimuli over the three week period in the control group, in which there was no change in the plasma zinc concentration level of the subjects. Furthermore, these changes in taste responses were not significantly different from the changes shown by the subjects in the experimental group whose plasma zinc levels rose significantly during the two weeks of treatment with zinc sulphate. These findings seem to dismiss the hypothesis that zinc metabolism plays a role in taste processes.

It is conceivable that the plasma zinc concentration level of the patients at the time of admission to the hospital reflects the overall physical health state of the patients. The lower

than normal plasma zinc level may represent a nutritional deficiency state of the patients which is common in alcoholics, as discussed previously. There are evidences that several nutritional elements affect taste processes, as in the case of Vitamin A. Bernard et al. (1961) showed that after prolonged Vitamin A depletion, rats showed a decrease in degree of rejection of quinine sulphate solutions with increased rejection of sodium chloride towards the end of the depletion. Vitamin A injections resulted in normal responses to sodium chloride.

It is generally held that after a period of abstention from alcohol, together with a balanced diet and multivitamin medication, the physical health of the alcoholic patients improves rapidly after their admission. It is probable that this improvement in overall physical health results in significant changes in taste perception over the three week period shown in this experiment. While low plasma zinc levels may indicate overall nutritional deficiency, improvement in other nutritional states does not necessarily result in increases in plasma zinc levels. It is common for the otherwise nutritionally adequate person to be deficient in zinc, for example, in the case of chronic leg ulcers patients. Therefore, it is quite conceivable that there are other factors, nutritional or otherwise which affect taste processes in this case besides zinc metabolism.

The findings that there were significant changes in responses to taste stimuli over the first three weeks of hospitalisation among alcoholic patients treated with placebos is contrary to the results of experiment Alcoholic Taste II reported earlier in this chapter. In that experiment, there was no significant change in responses to glucose, sodium chloride, citric acid and quinine sulphate of the same concentrations used

in this experiment over the same length of time. Besides the fact that there was no treatment of any kind given to the patients in the previous experiment for their taste alterations, the other point to be noted is that the concentration of solutions which the subjects correctly recognised were much higher in experiment Alcoholic Taste II than the ones in this experiment (see Table V.6 and Table V.9). This difference is unexplainable at this stage. It is unfortunate that no information on plasma zinc levels of the patients in experiment Alcoholic Taste II was known which prevents further meaningful comparison. Whether the initial level of taste functioning has any determining effect on improvement of taste perception is not known.

In the analysis of the data from this experiment with MANOVA, age had also been considered as a covariate and was found to have no significant effect on the responses to taste stimuli of any quality.

The aim of including zinc sulphate solution in the range of stimuli used in this experiment was to investigate the effect that zinc metabolism might have on the taste of a zinc compound, along similar lines to the effect of blood sugar levels on the perception of sweetness. The results from this experiment indicated that there were significant increases in perception of dryness i.e. there seemed to be a decrease in threshold to the "dryness of the mouth" aspect of the taste of zinc sulphate over the three week periods in both the experimental and control group. This finding seems to rule out the suggestion that zinc metabolism of the body has a significant effect on the perception of zinc sulphate solutions.

The zinc sulphate solutions were good indications of the reliability of the responses among the subjects of this experiment, in that they gave the experimenter an opportunity to assess the

attention of the subjects. All the subjects reported in this experiment, only recorded "dry" as responses to solutions of zinc sulphate, even though the solutions were presented to them at ramdom and they were not aware of the quality of each sample. This seems to indicate that the subjects responded according to what they perceived and did not respond at random.

As reported earlier, alcoholic patients showed a common confusion in the perception of saltiness and sourness, i.e. they commonly responded to sodium chloride solutions at the concentrations of 9.375% and lower as being sour. The correct recognition of solutions at higher concentrations ruled out the possibility of this confusion being a problem of nomenclature as reported by Myers (1904,1905). This confusion has not been reported previously in the literature although Gregson (personal communications) found in his unpublished study of taste in diabetics that there were some atypical taste confusions among his subjects. This phenomenon cannot be explained at this stage.

The subjects in both control and experimental groups were given placebos during the first week of this experiment. This was to avoid interference with other medications given during this period. Also, this period served as a screening period for patients who complained of the side effects of medication. None of the patients who actually received zinc medication reported or showed signs of side effects while five patients were excluded from the experiment when they complained of dizziness, lethargy which they attributed to the placebos given to them during the first week of this experiment.

The fact that there were changes in objective taste recognition among the patients in both experimental and control groups during the first week of hospitalisation while they only

received placebo medication was a further evidence against the hypothesis that zinc metabolism has significant effects on taste processes.

The overall results from this experiment seem to indicate that zinc metabolism did not play a significant role in taste processes among the alcoholic patients. This result is contradictory to the hypothesis proposed by Henkin and his colleagues as discussed in Chapter IV of this thesis.

Before further conclusions are drawn, it may be appropriate to consider the limitations of this experiment.

Firstly, the small number of subjects included in this experiment is open to criticism. However, out of ninety-five patients admitted to the Alcoholic Unit over a six-month period, forty patients who reported subjective decrease in taste sensitivity agreed to participate in this experiment. Out of these forty, five had to be excluded due to complaints of side effects during the treatment with placebos in the first week of study. Ten other patients discontinued with the experiment at different stages of the study through either being discharged or having left the hospital against medical advice. The twenty-five subjects reported in this study were the available subjects who participated voluntarily right through the whole experiment.

Secondly, as discussed earlier, the stimuli used in this experiment were selected in pilot studies. The limited attention span found in alcoholic patients prevented the employment of a bigger range of stimuli. The concentration steps between stimuli may be criticised as being too large, but these stimuli seemed to be sensitive enough to allow for the study of changes in taste perception.

Thirdly, the above reason also put a limit on the number

of trials that can be carried out in each testing session. Each solution was presented only once in one session, an unacceptable procedure in the determination of thresholds. In this experiment, the aim was not to measure thresholds per se but to study the changes in responses to stimuli over a period of time. The randomisation of stimulus quality, while keeping concentrations in ascending order in each series together with water blanks and the introduction of zinc sulphate solutions into the range of stimuli used, seemed to produce a satisfactorily reliable set of data. Several checks had been carried out on the reliability of the responses to the stimuli of the subjects by representing some solutions without the knowledge of the subject. The rate at which the same response was given to the same solution in separate trials in one session was very high (82 per cent).

Fourthly, lack of facilities and personnel prevented extensive medical examination to be carried out to ensure no medical or physical complications which may affect taste perception. These detailed medical examinations and laboratory analyses are the strongest points in the work of Henkin and his colleagues. Ideally, in this study, plasma zinc levels of each patient should have been obtained at regular intervals right through the three-week period, or at least on the days which the taste assessments were to be carried out. This would have allowed further investigation on the relationship between plasma zinc levels and taste sensitivity.

Finally, a common criticism of a study of this nature would be that a double blind study is preferable to a single blind study. It was not possible from the practical point of view to carry out a double blind study in this situation since the experimenter was not a permanent staff member of the Alcoholic

unit. The administration of medication and the management of the subjects had to be done by the nursing staff of the unit who were not in a position to participate in a double blind study.

In this experiment, the experimental design was more rigidly controlled than the conventional single blind situation. The subjects were not aware of the type of medication given to them and the effects that the medications may have on their taste functioning. The medication was administered by the nursing staff who were instructed to distribute the medications to the subjects according to a chart, where the medications were labelled as Medication G (placebos) and Medication Z (zinc sulphate). The instructions given to the subject during the taste assessment prevented the experimenter from influencing the responses of the subjects. The choice of method of testing minimised the bias problems which were the disadvantages of the drop method discussed earlier. This is in contrast to the method used in the single blind study of Schechter et al. (1972) who used the drop method of testing. In their experiment, the subjects were admitted to the hospital for treatment of their taste abnormalities, the medications were administered by the experimenters themselves, while the subjects expected the medications to improve their taste sensitivity, which clearly increased the bias problems.

IV. CONCLUSIONS

Bearing the above limitations in mind, we can conclude from the results of this series of experiments that

(1) Zinc sulphate medication at the dosage of 660 mg $ZnSO_4$ (150 Zn^{++}) daily raised serum and plasma zinc levels from the previously deficient levels to above normal levels within the first

two days of administration. This dosage of zinc sulphate did not produce any side effects among the chronic leg ulcers and alcoholic patients. Serum and plasma zinc levels remained high above the normal level so long as the zinc therapy was continued.

(2) The alcoholic patients in general and those alcoholic patients who complained of subjective decrease in taste sensitivity in particular, had plasma zinc levels below the normal value. Without zinc therapy, the plasma zinc levels did not return to normal during the first three weeks of hospitalisation.

(3) Alcoholic subjects correctly recognised solutions of glucose, sodium chloride, citric acid and quinine sulphate at concentrations higher than normal subjects of the same age group, indicating an objective decrease in taste sensitivity.

(4) Without medication, this decrease in taste sensitivity did not change significantly during the first three weeks of hospitalisation.

(5) When alcoholic subjects were given either placebos or zinc sulphate medication, there were significant changes in responses to taste stimuli indicating improvement in taste sensitivity during the first three weeks of hospitalisation.

(6) There were no significant differences between the results obtained from subjects in the experimental and control groups which indicated that zinc metabolism did not play a significant role in the taste processes of these subjects. This is in contradiction to the earlier findings of Henkin and his associates.

CHAPTER VI

EXPERIMENTS ON THE TASTE OF ZINC SULPHATE AND THE EFFECTS OF ZINC SULPHATE MOUTHRINSE ON TASTE SENSITIVITY

In using zinc medication for the treatment of chronic ulcers, there are evidences that zinc is effective both when administered orally as zinc sulphate and when applied topically in the form of ointments and salves. Even though zinc has been claimed to play an important part in taste processes and the oral administration of zinc sulphate has been reported to improve taste sensitivity (Schechter et al.1972), there has been no study undertaken to investigate the possible action of zinc sulphate applied topically in the oral region as a mouthrinse. If the hypothesis proposed by Henkin that a zinc containing protein exists in the saliva which regulates the growth and nutrition of the taste buds is true, the application of zinc sulphate as a mouthrinse may have some effect on taste perception.

A series of experiments had been conducted to obtain some preliminary information into the use of zinc sulphate as a mouthrinse and its effect on taste perception. It must be noted at the outset that these experiments are exploratory in nature and can only be treated as pilot studies.

To be used as a mouthrinse zinc sulphate crystals were dissolved in deionized water at different concentration levels. Before these solutions can be used as a mouthrinse, the characteristic taste of the solutions themselves must be established. In a simple trial, zinc sulphate solutions at the concentrations between .01 and 1.0 percent, weight by volume, produced a variety of tastes reported by the subjects who took

part, including a mixture of sweet, salt, sour and bitter with the sensations of dryness of the mouth and prickliness on the tongue and other oral surfaces. The variation in responses indicated that a detailed investigation of the exact taste of zinc sulphate at various concentration levels must be carried out before the solutions could be used as mouthrinses.

This chapter contains the descriptions and results of a series of experiments on the taste of zinc sulphate solutions and the pilot study into the use of zinc sulphate solution as a mouthrinse.

I. EXPERIMENT ZINC SULPHATE TASTE I

One method of investigating the effects of a mouthrinse on taste perception is to present a solution (X) to a subject instructing the subject to remember the intensity and quality of the taste he perceives from that solution X, then to ask the subject to rinse his mouth with the mouthrinse in question and present another sample of solution X to the subject again (without the subject being aware that the first and second solutions are the same), while asking him to report the quality and intensity of the taste of this second solution in comparison to the first solution.

In this situation, there are three factors to be investigated.

(a) The characteristic taste of the mouthrinse itself.

(b) The change in taste quality of the testing solution, (in this case the second sample of solution X) due to the mixing of the mouthrinse and the testing solution itself in the oral cavity.

(c) The change in perceived intensity of the testing solution reported by the subject after the mouthrinse, i.e. whether the mouthrinse enhances or suppresses the intensity of the testing solution.

All these changes presuppose some fixed time schedules of successive tastings.

The perceptual changes that occur when chemicals are tasted in mixture solutions have been well documented. Moskowitz (1972) reported that sweet mixed with either sour or bitter blended in almost all proportions. The "flavour" of sweetness in mixtures differed from that of simple sugar sweetness, which suggested that the presence of a second taste modified the qualitative aspect of sweetness. Gregson (1966) found that near-threshold mixtures of sucrose and sodium chloride elicited acidic or bitter sensations as well as, or instead of, sweetness and saltiness.

To investigate perceptual changes that may occur due to the mixing of zinc sulphate solutions used as mouthrinses and the testing solutions in the oral cavity, an experiment was conducted. This experiment studied the taste of mixture solutions of zinc sulphate and sucrose at various concentrations and determined the effect of zinc sulphate on the taste of sucrose.

(a) Subject. Ten male and ten female undergraduate third year psychology students, took part in this experiment. None of the subjects had any previous experience of taste experimentation. They took part in this experiment as part of their course exercises.

(b) Method. The stimuli used were nine mixtures of sucrose and zinc sulphate in varying concentrations. Three concentrations of each chemical were used. The stimulus series is schematically presented in Table VI.1.

TABLE VI. 1. STIMULUS SERIES USED IN EXPERIMENT ZINC SULPHATE TASTE I

Sucrose Conc. (mg/ml)	ZINC SULPHATE CONCENTRATION (mg/ml)		
	0	0.1	0.2
0.5	1	2	3
1.0	4	5	6
2.0	7	8	9

All mixtures were dissolved in deionized (equivalent to triple distilled) water with resistance not less than 2MΩ/cm. Stimuli were 30 ml samples in 50 ml beakers kept at room temperature.

The experiment was carried out as a class exercise in a group situation, in that all subjects were seated in the same room but without communication between them. Each subject was assigned a code number and presented with a random series of the nine mixture stimuli.

The subjects were asked to taste the solutions in front of them in succession and to record their responses on provided data sheets. (Appendix II) The subjects were instructed to consider their responses according to 8 taste qualities, sweet, salt, sour, bitter, acid, dry, prickly and water, the qualities used to describe the taste of zinc sulphate by subjects of an earlier preliminary study. They were asked to rate the intensity of each quality perceived in any one solution along an eleven point scale of intensity. The scale was; Extremely Strong, Very Strong, Strong, Fairly Strong, Intermediate, Weak, Very Weak, Faint, Just Detectable, Uncertain, Not Detectable.

In this manner each solution had eight ratings, one in each of the eight taste quality scales according to the sensations perceived by the subject. Three replication trials

of the whole series were performed by each subject. The formal instructions to the subjects were as follows:

"In this experiment, you will be required to taste harmless solutions and record your experiences in the provided sheets according to their tastes and intensities.

The solutions are presented in beakers in front of you. When instructed to do so by the experimenter, you are to sip one third of the solution from the beaker, one at a time starting from your left. Take it into your mouth and taste it, making sure you roll it around your tongue, record your experience and then swallow the solution. You will be advised of the time to taste the following solution. Use one record sheet for each solution.

You are to repeat this procedure three times, so make sure you only take one-third of the solution in your beaker at a time. Also record your subject number and sample number on each record sheet."

There was a one-minute time interval between the tasting of two samples and a five-minute interval between trials. During each interval between trials, the record sheets of the previous trial were removed to prevent subjects referring to them in the new trial.

(c) Result and Discussion. The rating in each quality assigned by the subject to a solution was given a score ranging from 1 for "not detectable" to 11 for "extremely strong". In this way each stimuli yielded a series of 8 rating scores, one for each scale considered. The mean and standard deviation values of these rating scores had been computed for each stimuli in each trial series and compared with those from the other two trial series. Table VI.2 presents the mean and standard deviation values of rating scores for stimuli in trial I.

TABLE VI. 2. MEAN AND STANDARD DEVIATION VALUES OF RATING SCORES FOR STIMULI IN TRIAL I OF EXPERIMENT ZINC SULPHATE TASTE I

SOLUTION NUMBER *		SWEET	SALT	SOUR	BITTER	ACID	DRY	PRICKLY	WATER
1	$\bar{X}$	1.524	1.237	1.277	1.490	1.293	1.590	1.140	2.738
	S.D.	0.671	0.423	0.531	0.710	0.491	0.704	0.399	0.728
2	$\bar{X}$	1.384	1.550	1.987	2.120	1.508	2.242	1.553	1.968
	S.D.	0.594	0.723	0.890	0.893	0.670	0.742	0.787	0.874
3	$\bar{X}$	1.335	1.709	2.344	2.362	1.936	2.438	1.604	1.576
	S.D.	0.579	0.711	0.831	0.809	0.728	0.729	0.709	0.701
4	$\bar{X}$	2.127	1.487	1.366	1.507	1.374	1.434	1.146	2.338
	S.D.	0.805	0.573	0.626	0.753	0.517	0.626	0.414	0.780
5	$\bar{X}$	1.329	1.667	0.270	2.211	1.713	1.993	1.314	2.104
	S.D.	0.495	0.581	0.685	0.734	0.771	0.845	0.428	0.777
6	$\bar{X}$	1.221	1.747	2.345	2.440	1.870	2.445	1.472	1.550
	S.D.	0.470	0.686	0.689	0.724	0.691	0.667	0.674	0.647
7	$\bar{X}$	2.660	1.238	1.324	1.289	1.231	1.514	1.078	2.164
	S.D.	0.629	0.419	0.456	0.550	0.376	0.614	0.200	0.702
8	$\bar{X}$	1.931	1.447	1.864	1.971	1.530	2.066	1.279	1.913
	S.D.	0.774	0.518	0.904	0.897	0.732	0.907	0.527	0.642
9	$\bar{X}$	1.705	1.949	2.280	2.277	1.850	2.504	1.586	1.608
	S.D.	0.757	0.691	0.761	0.805	0.845	0.671	0.593	0.619

* solution number as shown in Table VI.1

The multivariate analysis of variance, MANOVA, had been performed on this set of data in order to test for significance of the following factors:

Factor S. The sucrose concentration of the stimuli, to see whether the sucrose concentration values had any significant

effect on the rating scores of the stimuli.

Factor Z. The zinc sulphate concentration of the stimuli, to see whether the zinc sulphate concentration values had any significant effect on the rating scores of the stimuli.

Factor R. The replication effect, to see whether there were significant differences among the rating scores of each stimuli in the three trials.

Factors SZ, SR, ZR, SZR. The interaction effects, to see whether the combinations of the above three factors had any significant effect on the rating scores of the stimuli.

In these analyses, the criteria variables were the rating scores of the eight qualities considered. Several variables had been considered as covariates. In the test of within cells regression, the sex of the subjects and the ratings of the immediately preceding stimulus were found to be significant as covariates to the criteria variables. This finding indicated that the rating in the scales of sweet, bitter, sour and dry, which were the scales investigated, of one stimulus had significant effects on the rating scores of the immediately following stimulus.

Taking the sex of the subjects and the prior ratings of sweet, bitter, sour and dry as covariates, the MANOVA had been performed and the results summarised in Table VI. 3.

TABLE VI. 3. SUMMARY TABLE OF THE RESULTS FROM MULTIVARIATE ANALYSIS OF VARIANCE FOR DIFFERENT FACTORS

Factor	Number of Roots	Range of F Values	Number of significant roots	Range of Canonical Correlation (R) Values
S	2	9.338 - 1.081	1	0.481 - 0.122
Z	2	17.099 - 0.674	1	0.614 - 0.097
R	2	0.351 - 0.274	nil	0.086 - 0.062
SZ	4	1.290 - 0.481	nil	0.220 - 0.069
SR	4	0.565 - 0.204	nil	0.122 - 0.045
ZR	4	0.758 - 0.448	nil	0.145 - 0.067
SZR	8	0.750 - 0.033	nil	0.183 - 0.008

The univariate analyses showed that zinc sulphate concentration had significant effect ($p < .001$) for all the eight taste qualities considered while the sucrose concentration had significant effect only on the ratings of sweet ($p < .001$) and water ($p < .046$).

Summarily, it was found that there were no significant differences in the ratings of stimuli over the three trials, indicating that the ratings were replicable in this experimental condition. There were no significant interaction effects of any combination of factors. The concentration of zinc sulphate in the stimuli was significant in determining the ratings in all eight taste scales investigated and the sucrose contents of the stimuli were significant in the ratings of sweet and water. Looking at Table V.2, it could be said that the ratings of sweetness increased with the increasing concentration of sucrose in the solutions, while the ratings of water decreased.

The results of the analyses seemed to indicate that when

the subjects were asked to taste a mixture of zinc sulphate and sucrose, the subjects were able to rate the intensity of the two component chemicals separately. Zinc sulphate by itself had been found in pilot studies to elicit the sensations described by the eight taste qualities used as scales in this experiment.

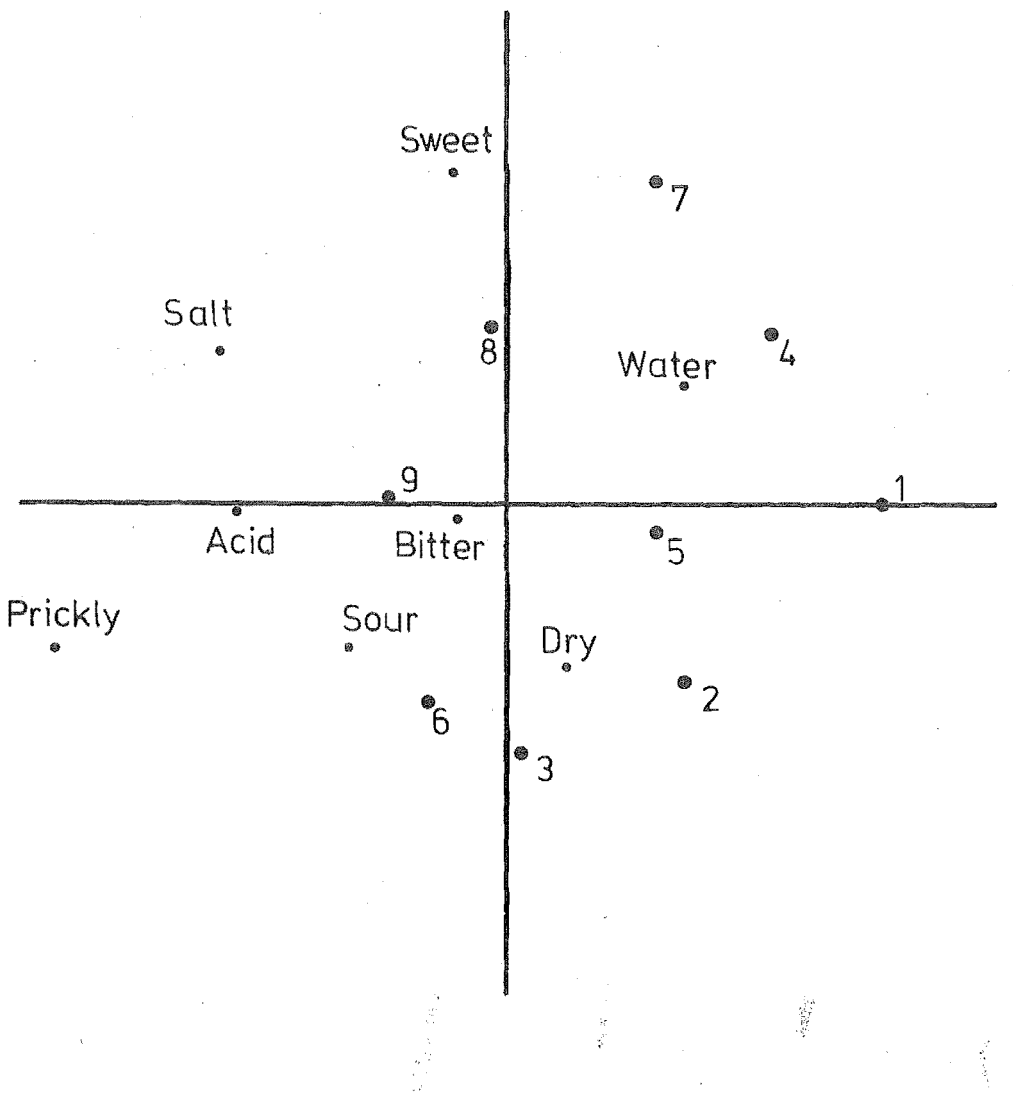
In the analysis of within cells correlation of criteria variables, it was found that there were no significant correlations between any of the eight scales used. This led to the assumption that all the scales were independent of each other. There seemed to be no difficulties in differentiating sour, bitter and acid, the commonly encountered problem in this type of experiment, among this group of subjects.

The MANOVA analyses failed to show any significant interaction effects of sucrose and zinc sulphate on the sensations perceived by the subjects. This prevented any meaningful conclusions to be drawn concerning the resultant taste of the mixtures. This factor was further investigated with the use of Multidimensional scaling method of analysis.

The multidimensional scaling of the data from this experiment was performed with the assistance of Mr. C.O. Fraser of the Psychology Department, University of Canterbury, using the POLYCON II program. The ratings from each subject were averaged over the three trials and arranged into a 9 x 8 matrix (9 solutions, 8 taste scales). The twenty individual matrices were then combined into a single data matrix (using the RMS).

The multidimensional scaling of this pooled data showed an excellent reproduction of the expected stimulus configuration. The rating scale points were located in approximately the positions which we would expect from prior knowledge of the contents of the stimuli. Sweet was in the direction of increasing

FIG. VI. 1 SCHEMATIC REPRESENTATION OF THE RESULT OF MULTIDIMENSIONAL SCALING ANALYSIS ON THE DATA FROM EXPERIMENT ZINC SULPHATE TASTE I



The numbers are stimulus labels as used in Table VI. 1

sucrose concentration. Dry, bitter and sour were located close to the high zinc sulphate concentrations. Water was near the points low on both dimensions, acid and salt near those high on both. Prickly was located further away than the other scales. These findings are schematically presented in fig.VI.1.

The results of this analysis indicated that there were some interaction effects between the two components, the concentrations of sucrose and zinc sulphate. However, in this situation, it seemed that the subjects were able to rate a mixture solution with scale values proportional to the concentrations of the components of the mixture, i.e. rating the sweet scale according to the concentration of sucrose while rating dry, bitter and sour according to the concentration of zinc sulphate in the same mixture.

The overall result indicated that the method and the type of scales used in this experiment were appropriate and valid in this situation. Each of the eight taste qualities used in the scale was independent of the others and the rating system was successful in that the subjects were able to rate the eight tastes on the same scale of intensity. The fact that no standard had been provided for the subjects to base their rating of intensity scale on, did not create any problems for the subjects in using the scale. S.S. Stevens (1969) in fact, suggests that in many cases in psychophysical studies, the designation of a standard is neither necessary nor advisable. By allowing the subject to choose his own modulus, the experimenter removes one more of the constraints that may otherwise produce minor distortions in the results.

From this experiment, we learned that if sucrose solutions were to be used as testing solutions in the mouthrinse

experiments described earlier, the mixture of sucrose and zinc sulphate in the oral cavity could elicit a complex sensation which resembled the combination of the tastes of sucrose and zinc sulphate themselves. However, the subjects were capable of rating the sucrose content of the mixtures which indicated that in a mouthrinse experiment, one would be able to ask "which of the two solutions (the pre-rinse testing solution and the post-rinse testing solution) is sweeter".

II. EXPERIMENT ZINC SULPHATE TASTE II

It was clear from the previous experiment and pilot studies, that zinc sulphate solutions elicited a complex combination of taste sensations. Before these solutions can be used as mouthrinses, further investigations must be carried out to establish the taste of zinc sulphate at different concentrations in the range that may be used as mouthrinses.

Also, from the psychophysical point of view, it is interesting to find out whether zinc sulphate belongs to the class of chemicals which do not elicit the same qualitative sensations as their physical concentrations in aqueous solutions are progressively increased. Gregson (1969) reports that sodium benzoate may taste at first salty, then sweet, then bitter, as its concentration is progressively increased. At high concentrations, sodium benzoate has a flavour which some subjects describe as scented or oily. At any given concentration it can evoke different qualitative responses from different subjects, and it can readily evoke different responses from the same subject on successive trials.

For these purposes, an experiment was conducted to study the taste sensations elicited by a series of zinc sulphate

solutions of various concentrations.

(a) Subjects. The subjects were 24 undergraduate Psychology students who had no previous experience of taste experiments and were naive about the nature of this experiment. Each subject was paid one dollar for taking part in this experiment. There were 12 male and 12 female subjects whose ages ranged between 17 and 22 years old.

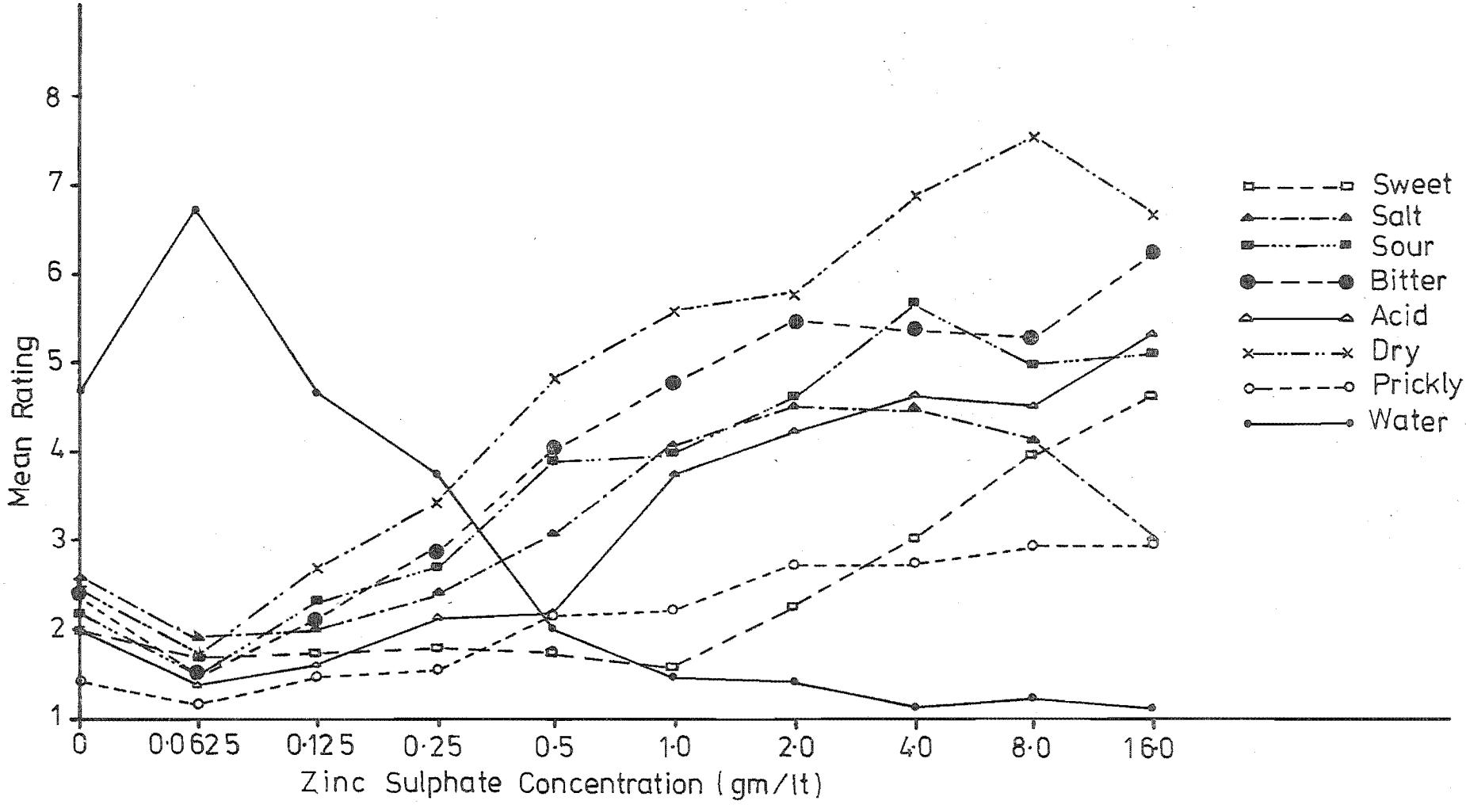
(b) Method. The stimuli used in this experiment were 9 solutions of zinc sulphate and one water blank. The concentrations of zinc sulphate solutions were 0.0625, 0.125, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0 and 16.0 gm/lt. All solutions were dissolved in deionized water with resistance of not less than 2 MΩ/cm. Stimuli were 30 ml samples in 50 ml beakers kept at room temperature.

The experiment was conducted in a group situation similar to that in Experiment Zinc Sulphate Taste I. The subjects were divided into 3 groups of 6 subjects and each group took part in one of 3 identical sessions. The experiment took place in the Physiology Laboratory at the University of Canterbury.

Each subject was asked to rate a series of 10 samples on the same scales as used in Experiment Zinc Sulphate Taste I (see Appendix II for a sample of the scales used). The nine zinc sulphate solutions were presented in ascending order of concentration and the water blank samples were in randomised positions in the stimulus series. Three replication trials were obtained from each subject. There were 20-second intervals between each sample and 2-minute intervals between each trial.

The subjects were instructed to record their taste sensations arising from each stimulus on the scales, while holding the stimulus in their mouths. They were also told to expectorate

FIG. VI. 2 POOLED MEAN RATINGS ON DIFFERENT SCALES OF THE TASTES OF ZINC SULPHATE SOLUTIONS.



and rinse their mouths with tap water ad lib.

The formal instructions to the subjects were exactly the same as those used in Experiment Zinc Sulphate Taste I except for swallowing the solutions.

(c) Results and Discussions. The ratings were coded in the same way as the previous experiment. The mean and standard deviation values of ratings on each of the eight scales, for each solution, in each trial were calculated from the results obtained from the twenty-four subjects. The mean ratings for each solution were then pooled over three trials and presented graphically in fig. VI.2.

MANOVA analyses had been performed on the data from this experiment to test for significance in the following factors.

Factor Z. The zinc sulphate concentrations, to see whether the concentration of zinc sulphate in the samples had any significant effects on the rating of the scales.

Factor R. The replication effect, to see whether there were significant differences among the ratings of each solution in the three replication trials.

Factor ZR. The interaction effect of the above two factors.

In the analyses, several variables had been considered as covariates. It was found that the significant covariates were sequence and prior ratings of sweet, salt, sour, bitter and dry. Sequence was the position which a stimulus occupied in the stimulus series presented to each subject. Even though the zinc sulphate solutions were presented in ascending order of presentation, the water blank sample was randomly placed in the stimulus series, which caused the variation in sequence. The significant effects as covariates of prior ratings of sweet, salt, sour, bitter and dry were in the same manner as discussed in the

previous experiment. The criteria variables in these analyses were ratings in the eight scales used in the experiment.

Taking sequence and prior ratings of sweet, salt, sour, bitter and dry as covariates, the MANOVA analysis was performed on the data and the results summarised in Table VI.4.

TABLE VI.4. SUMMARY TABLE OF THE RESULTS FROM MANOVA ANALYSIS OF THE DATA FROM EXPERIMENT ZINC SULPHATE TASTE II

Factor	Number of Roots	Range of F Values	Number of significant roots	Range of Canonical Correlation (R) Values
Z	8	4.357 - 0.141	2	0.496 - 0.020
R	2	0.402 - 0.234	nil	0.084 - 0.049
ZR	8	1.055 - 0.164	nil	0.276 - 0.051

It was clear that factor Z was the only significant factor. The results of univariate F test for the criteria variables for factor Z are tabulated in Table VI.5.

TABLE VI.5. UNIVARIATE F TESTS FOR ALL CRITERIA VARIABLES FOR FACTOR Z (EXPERIMENT ZINC SULPHATE TASTE II)

VARIABLE	F(9,684)	MEAN SQUARES	P LESS THAN
SWEET	2.402	12.246	0.011
SALT	5.895	36.766	0.001
SOUR	5.547	48.697	0.001
BITTER	8.110	76.861	0.001
ACID	4.781	39.075	0.001
DRY	12.496	103.849	0.001
PRICKLY	1.901	10.900	0.049
WATER	9.619	80.930	0.001

Interpreting the results of the analyses, the results showed that the ratings of each stimulus were stable over the replication trials i.e. there were no significant differences in the ratings of all the eight sclaes over the three trials. There was no interaction effect between factors Z and R.

The concentration of zinc sulphate had significant effects on the ratings of the stimuli. Looking at fig. VI.2. it seemed that the ratings of water decreases as the concentration of zinc sulphate in the solutions increases. The ratings of water blank samples ($ZnSO_4$ concentration 0 gm/lt in the figure) were higher than the ratings of 0.0625 gm/lt zinc sulphate solutions, indicating that there were after effects of zinc sulphate affecting the ratings of water blanks which were positioned randomly in the stimulus series.

For zinc sulphate solutions with concentrations up to 4 gm/lt, the ratings of sweet, salt, sour, bitter, acid, dry and prickly increased as the concentration was progressively increased. There were some unusual interactions between the scales in 8.0 and 16.0 gm/lt solutions of zinc sulphate.

In conclusion, it can be said that zinc sulphate has a complex characteristic taste. It elicits a sensation of dryness of the oral cavity with a taste of sour, bitter, acid and underlying tastes of sweet and salt, together with a sensation of prickliness of the tongue. The quality of the taste of zinc sulphate does not change with increasing concentration while the intensity of different taste qualities elicited by the chemical, does.

Methodologically, the procedure employed seemed to be as valid in this situation as in the previous experiment. The subjects were able to use the rating scales without the provision

of a standard. The fact that the prickly scale showed the lowest F value in the univariate F test (Table VI.5) agreed with the findings of the multidimensional analyses discussed in the previous experiment. This indicated that prickly was the least descriptive of the stimuli of all the scales used.

III. EXPERIMENT ZINC SULPHATE TASTE III

In the previous experiment, it was seen that there were some unusual interactions between the scales used to describe the taste of zinc sulphate, at concentrations higher than 4 gm/lt. Looking at figure VI.2. in the mean ratings of dry, bitter, acid and sour, at 8 gm/lt, the zinc sulphate solution seemed to elicit a weaker sensation of bitter, acid and sour while the dryness sensation was higher than the immediately preceding solution of less concentration. The reverse was true when the concentration rose to 16 gm/lt.

It was not clear whether this unusual interaction of scales at these concentrations was a characteristic property of the taste of zinc sulphate or a distortion in the results of that experiment. It was considered of interest to conduct another experiment to investigate this phenomenon and also to establish whether the results of the previous experiment were replicable in another group of subjects.

(a) Subjects. Twenty-two undergraduate Psychology students took part in this experiment. Their ages ranged between 17 and 23 years old. There were 11 male and 11 female students. These subjects came from the same population of students as those who acted as subjects in the previous experiment. They had no previous experience of taste or other psychophysical experiments. They were paid one dollar each for taking part in the experiment.

(b) Method. The stimulus series used in this experiment had been extended to cover twelve stimuli, 11 zinc sulphate solutions and one water blank. The concentrations of zinc sulphate solutions were 0.0625, 0.125, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0, 64.0 gm/lt, all dissolved in deionized water.

The experiment was conducted in three identical sessions in the Physiological Laboratory at the Psychology Department, University of Canterbury. The subjects were divided into 3 groups each of which took part in one of the experimental sessions.

The order of presentation of the stimuli, the method of tasting, the instructions to the subjects and the scales used to record their responses were exactly the same as in the previous experiment. Three replication trials of the twelve stimuli were obtained from each subject.

(c) Results and Discussions. The ratings were coded and the means and standard deviation values of ratings on each of the eight scales, for each solution, in each trial were calculated in the same manner as described previously. The results showed the same trend as those presented in fig. VI.2.

MANOVA analyses were performed on the data to test for significance of factors; Z, the concentration of zinc sulphate solutions, R, the replication trials and ZR, the interaction effect. Sequence and the prior ratings of sweet, salt, sour, bitter, acid and dry were found to be significant covariates similar to those discussed in the previous experiment. The summary table of the results of multivariate tests of significance for the three factors is as follows:

TABLE VI.6. SUMMARY TABLE OF THE RESULTS FROM MANOVA ANALYSIS OF THE DATA FROM EXPERIMENT ZINC SULPHATE TASTE III

Factor	Number of Roots	Range of F Values	Number of significant roots	Range of Canonical Correlation (R) Values
Z	8	3.110 - 0.115	3	0.431 - 0.026
R	2	0.488 - 0.420	nil	0.085 - 0.066
ZR	8	1.122 - 0.246	nil	0.339 - 0.074

Again, factor Z was found to be the only significant factor in this experiment. The results of univariate F tests for all eight criteria variables for factor Z has been tabulated in Table VI.7.

TABLE VI.7. UNIVARIATE F TESTS FOR ALL CRITERIA VARIABLES FOR FACTOR Z (EXPERIMENT ZINC SULPHATE TASTE III)

VARIABLE	F(11,677)	MEAN SQUARES	P LESS THAN
SWEET	2.894	12.108	0.001
SALT	4.853	19.731	0.001
SOUR	5.224	29.328	0.001
BITTER	3.310	20.618	0.001
ACID	2.936	13.230	0.001
DRY	8.110	50.316	0.001
PRICKLY	2.828	14.197	0.001
WATER	4.714	36.604	0.001

We can conclude that the concentration of zinc sulphate had significant effects on the ratings of all eight scales used in this experiment. There were no significant differences among the ratings of solutions in the three replication trials (Factor R). There were no significant interaction effects of the factors Z and R.

Figures VI.3, VI.4, and VI.5 present graphically the

FIG. VI. 3 THE COMPARISON OF RATINGS ON THE "DRY" SCALE OF SOLUTIONS IN EXPERIMENTS ZINC SULPHATE TASTE II AND III

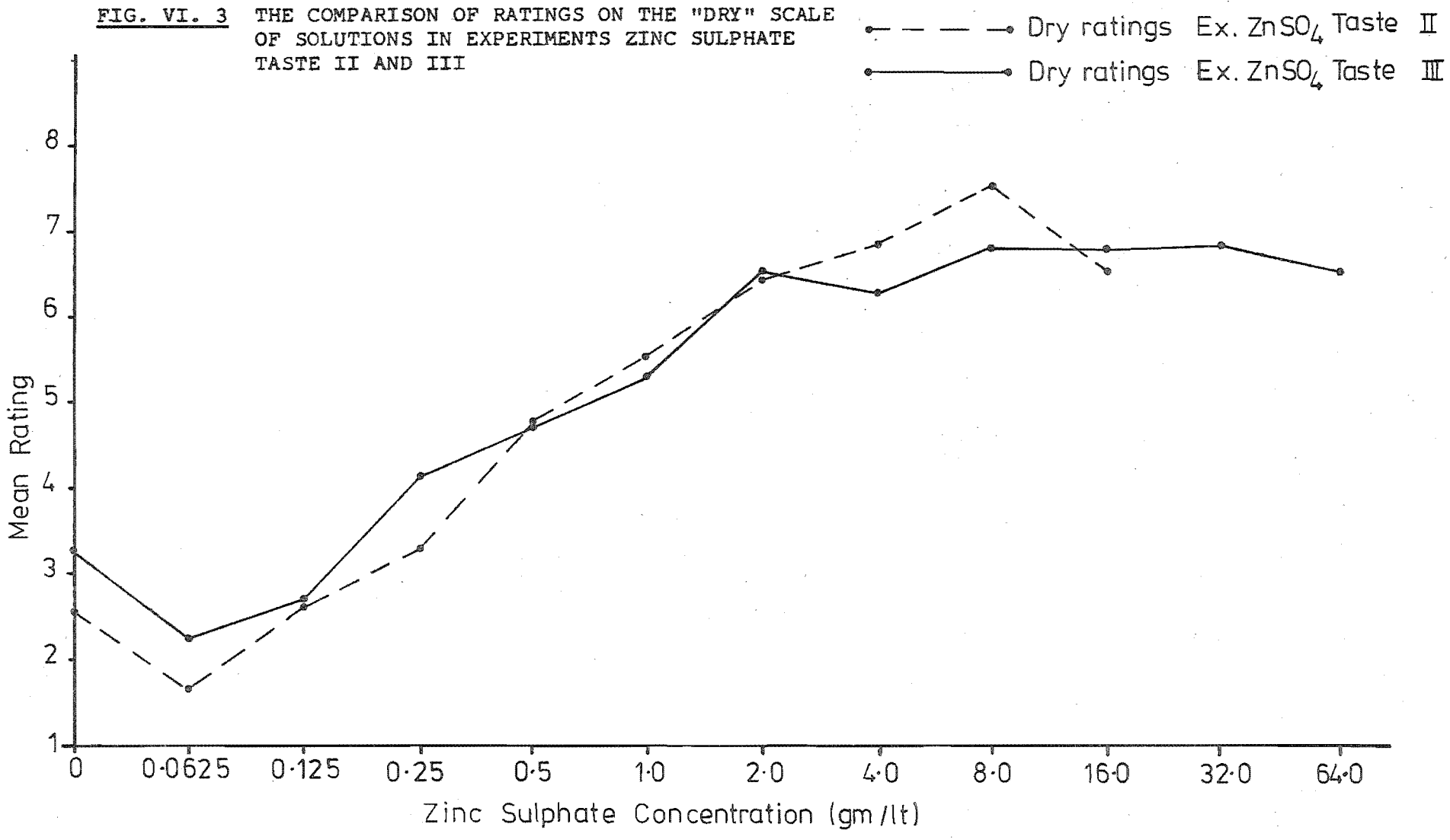


FIG. VI. 4 THE COMPARISON OF RATINGS ON THE "SOUR", "BITTER", "ACID" SCALES OF SOLUTIONS IN EXPERIMENTS ZINC SULPHATE TASTE II AND III

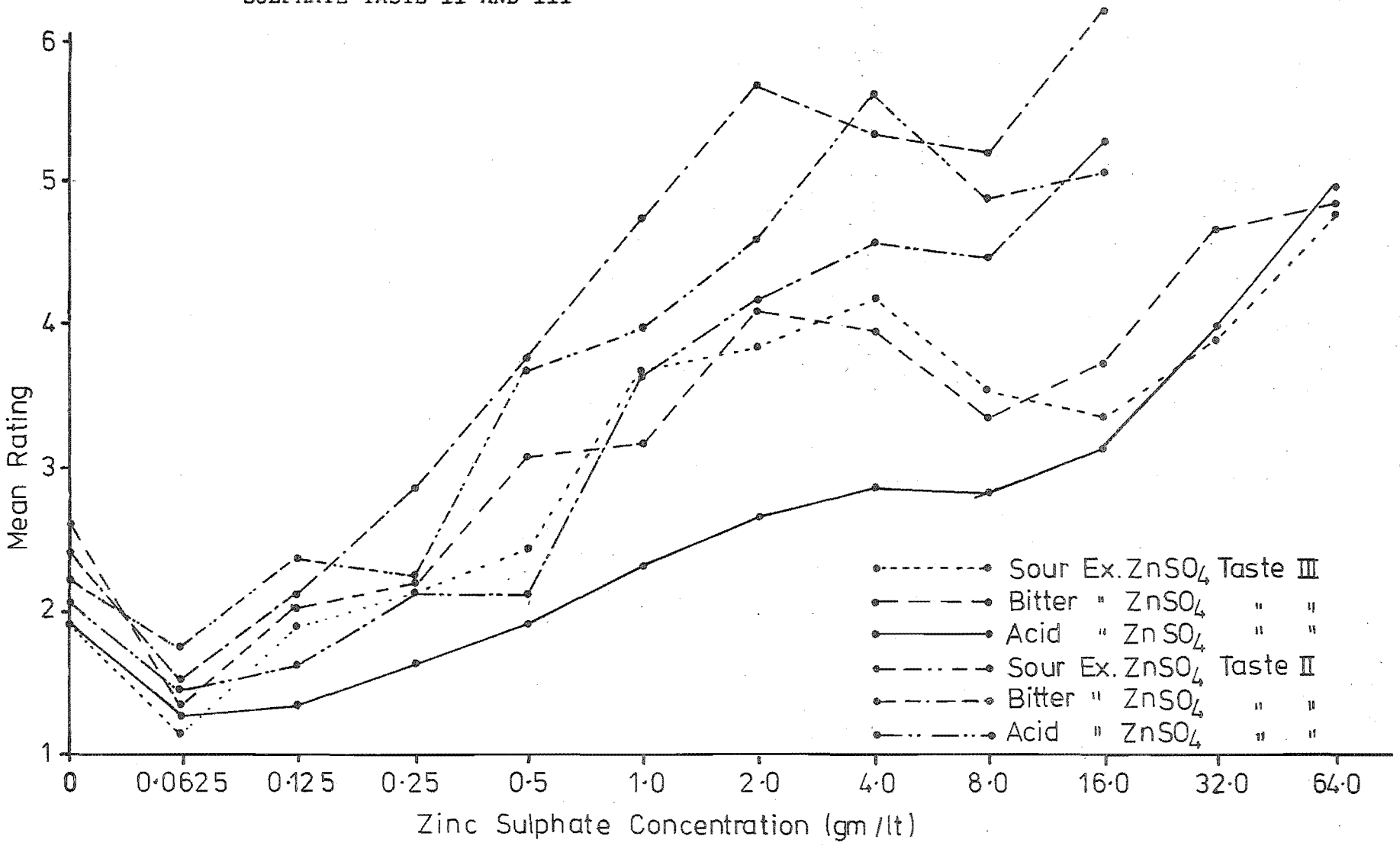
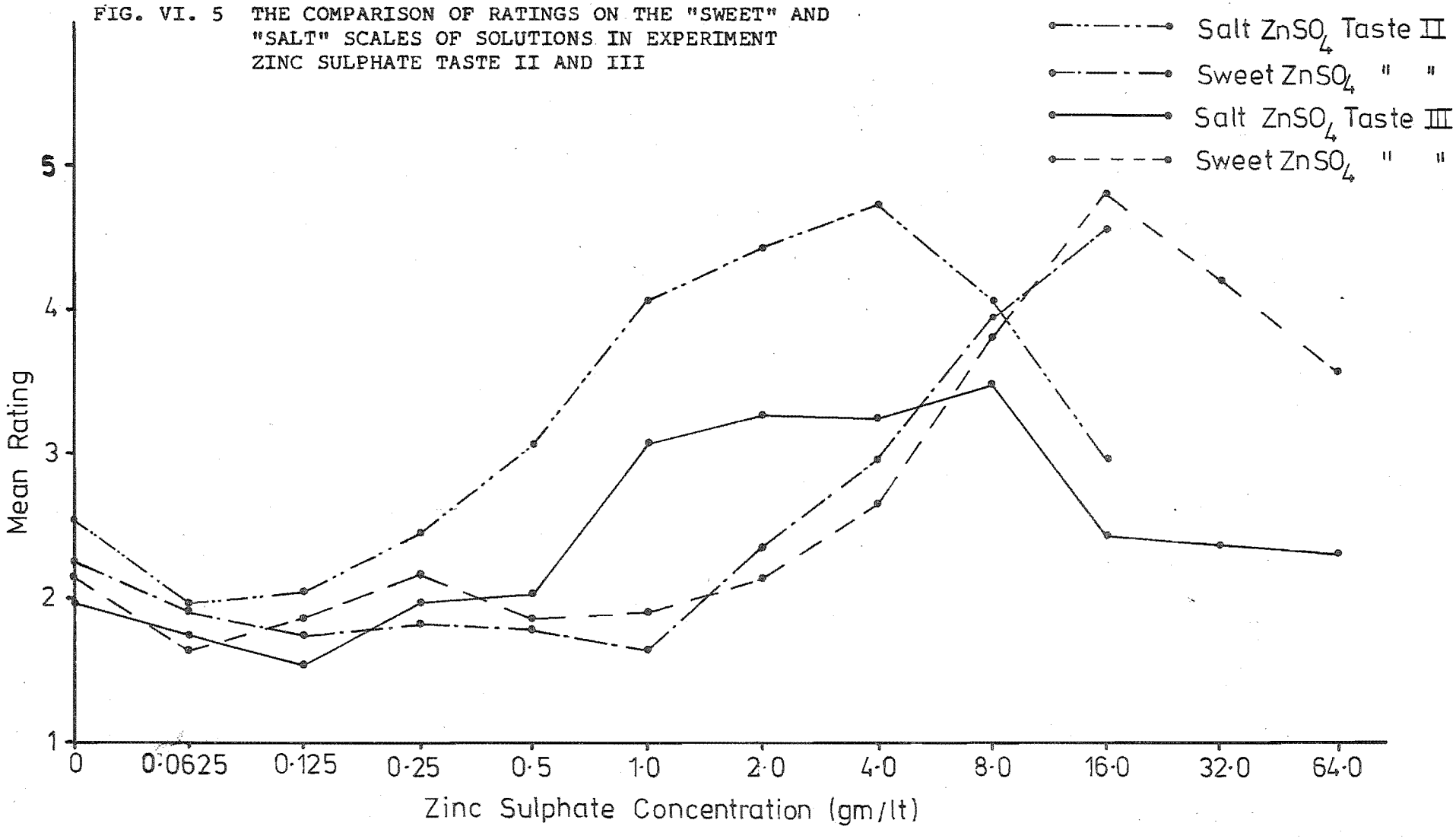


FIG. VI. 5 THE COMPARISON OF RATINGS ON THE "SWEET" AND "SALT" SCALES OF SOLUTIONS IN EXPERIMENT ZINC SULPHATE TASTE II AND III



comparison of mean ratings of the solutions used in Experiments Zinc Sulphate Taste II and III.

Clearly, it can be seen that the pattern of ratings in the seven scales shown in the graphs were similar in the two experiments. The ratings of all seven scales increases as the concentrations of zinc sulphate in the solutions progressively increases. The interaction between scales seen in the solutions of zinc sulphate at concentrations of 8 gm/lt and over, appeared in Experiment Zinc Sulphate Taste III in the same way as in the previous experiment.

Overall, the ratings of the ten solutions common in both experiments were higher in Experiment Zinc Sulphate Taste II than in Experiment Zinc Sulphate Taste III but the graphs were more or less stepwise parallel showing similar patterns of responses. A MANOVA analysis had been performed to compare the ratings of these ten solutions in the two experiments. Sequence and prior ratings of sweet, salt, sour, bitter and dry were significant covariates. The analysis showed a significant root ($p<.001$) in the multivariate test of significance. The univariate F tests showed that there were significant differences in the ratings of sour ($p<.012$), bitter ($p<.001$), acid ($p<.001$) and water ($p<.011$) between the data from the two experiments.

These significant differences in ratings between the two experiments seemed to be in actual rating scores only and not in the pattern of taste sensations perceived by the subject. Looking at figures VI.3, VI.4 and VI.5, it seemed as though the subjects "scaled down" their ratings to accommodate the extra two solutions added in Experiment Zinc Sulphate Taste III. The zinc sulphate solutions were presented in ascending order of presentation without the subjects being aware of the arrangement.

The way in which the data turned out indicated that the type of scales and method used in this experiment were useful and appropriate in this type of experimentation.

The results of this experiment confirmed the findings of the previous experiment. It was clearly shown that the results were replicable in different groups of subjects. The interaction of scales at higher concentrations of zinc sulphate i.e. the shifts in the curves of sweet, salt, sour, bitter and acid, while the dry rating continued to rise with increasing concentration, together with the fact that the dryness sensation was the dominating flavour of zinc sulphate solutions at lower concentrations, may be interpreted as indicating that at higher concentrations dryness sensation "overruled" all other components of the taste.

It could be concluded from the results of Experiments Zinc Sulphate Taste II and III that zinc sulphate solutions had a complex taste representing a combination of bitter, sour, acid, with underlying tastes of sweet and salt, and a strong sensation of dryness of the oral cavity. The intensity of each taste component perceived by the subjects increased as the concentration of zinc sulphate progressively increased. At much higher concentrations (8 gm/lt and higher), the sensation of dryness dominated all other taste components.

IV. ZINC SULPHATE SOLUTIONS AS MOUTHRINSES

From the results of the previous three experiments, we have established the tastes of zinc sulphate solutions at various concentrations. Also we have examined the tastes of the mixtures of zinc sulphate and sucrose solutions. We found that when a subject was asked to taste a solution containing a mixture of zinc sulphate and sucrose, the subject was capable of responding

to the taste of zinc sulphate and the taste of sucrose separately. These findings have provided enough information needed for the study into the effects of zinc sulphate as a mouthrinse, if sucrose solutions were used as testing solutions.

As far as the experimenter was aware, there has not been any report in the literature on experiments into the effect of mouthrinses on taste perception. Several methodological problems needed to be solved before any preliminary study in this area could take place.

First, how would one go about measuring the effects of a mouthrinse? In this particular case, we were interested in finding out whether a zinc sulphate mouthrinse had any effect on taste sensitivity and if it did, whether it suppressed or enhanced the sensitivity. It was decided that a simple method of conducting the experiment would be to ask the subject to complete a task involving 3 solutions in X_1 R X_2 arrangement. The subject was to taste a solution X_1 and remember the flavour and intensity of the flavour elicited by that solution, then to rinse his mouth out thoroughly with the solution R. After that the subject would be asked to taste solution X_2 and compare the intensity of the flavour elicited by X_2 with that of X_1. The subject was to indicate whether the flavour of X_2 was the same as or weaker or stronger than X_1. The subject was not aware that X_1 and X_2 were two samples of the same solution.

Obviously in this situation, there were chances that the subject would perceive that solution X_2 had a totally different flavour from that of X_1. In this particular study it was anticipated that sucrose solutions would be used in the places of solutions X_1 and X_2 and Experiment Zinc Sulphate Taste I was conducted to study the effects that zinc sulphate might have on

the flavour of sucrose solutions.

Secondly, what solutions of zinc sulphate should one use in a preliminary study? It was decided that two concentrations of zinc sulphate would be used in a preliminary study. It was felt that the solutions of zinc sulphate used should not be so concentrated as to elicit an overwhelming dryness sensation which could create problems in the tasting of the post-rinse testing solutions. Zinc sulphate solutions at 1 gm/lt and 2 gm/lt were chosen as both solutions had a predominantly moderately bitter taste, with a certain degree of dryness sensation which were not so strong as to be overwhelming to the subjects (see fig. VI.2).

Thirdly, some other solutions which could be used as mouthrinses were needed to act as controls in this experiment. Water was an obvious control but it was felt that some other solutions which had similar tastes to the two zinc sulphate solutions should also be used as controls. No other chemicals had been known to produce the dryness sensation while having similar tastes to zinc sulphate solutions. So the closest match to the taste of those zinc sulphate solutions would be to match the bitter component of their tastes. Several pilot studies were conducted with senior psychology students who had some experience in taste experimentations as subjects, in an effort to find some chemicals which, when dissolved in deionized water, had bitter tastes similar to zinc sulphate solutions. It was found from these studies, that a 0.5% solution of magnesium sulphate and a 0.0005% solution of quinine sulphate most closely resembled the two concentrations of zinc sulphate solutions both in quality and intensity of bitterness. Therefore, water together with solutions of 0.5% magnesium sulphate and 0.0005% quinine sulphate were considered appropriate as controls.

With the above methodological problems solved, a preliminary study into the effects of zinc sulphate mouthrinses on taste sensitivity was conducted.

(a) Subjects. Twenty-two undergraduate psychology students took part in this study. There were 8 male and 14 female students aged between 18 and 23 years old. These students had no previous experience of taste experiments and took part in this experiment voluntarily.

(b) Methods. The subjects were asked to complete a task as described above involving 10 sets of solutions of the X_1 R X_2 arrangement. The ten sets of solutions were X_m R_1 X_m, X_n R_1 X_n, X_m R_2 X_m, X_n R_2 X_n, X_m R_3 X_m, X_n R_3 X_n, X_m R_4 X_m, X_n R_4 X_n, X_m R_5 X_m, X_n R_5 X_n, where X_m = 1.25% sucrose solution, X_n = 2.5% sucrose solution, R_1 = 0.1% (1 gm/lt) $ZnSO_4$ solution, R_2 = 0.2% (2 gm/lt) $ZnSO_4$ solution, R_3 = deionized water, R_4 = 0.5% magnesium sulphate solution, R_5 = 0.0005% quinine sulphate solutions.

All solutions were dissolved in deionized water with resistance not less than 2 MΩ/cm.

Each of the solutions X_1, R and X_2 were 10 ml samples contained in 50 ml beakers arranged in a wooden tray which had fixed cut-out spaces to fit the three beakers in a row. The three beakers were labelled X_1, R and X_2 for ease of instruction to the subjects and identification of solutions.

The ten sets of solutions were presented to the subjects in a randomised order of presentation. The subjects were asked to taste solution labelled X_1 and to remember the flavour and intensity of the flavour. After expectorating or swallowing X_1, the subjects were asked to rinse their mouths thoroughly with R, holding R in their mouths for 5 seconds, then to expectorate. X_2 was tasted 10 seconds following that. The subjects indicated

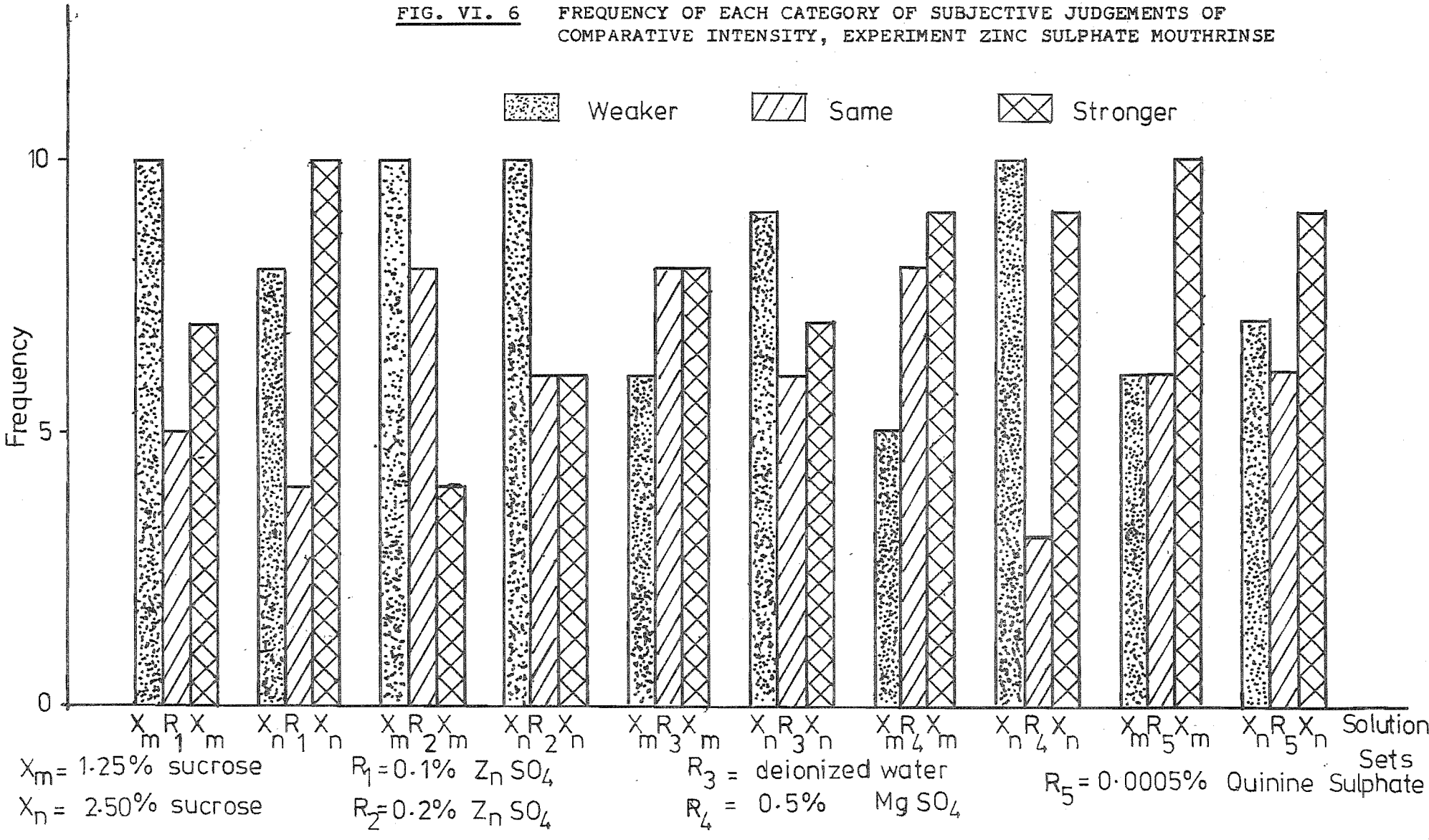
their responses by pointing to one of the three words "same, weaker, stronger" printed on a card placed in front of them. The subjects were asked to rinse their mouths out thoroughly with tap water after tasting each set of solutions. There were one-minute intervals between each set of solutions.

The subjects were seen in individual experimental sessions which took place in the Gustatory Psychophysics Laboratory.

At the beginning of the experiment, each subject was told that the experiment was designed to investigate the actions of different mouthrinses on taste functioning. He was told that the solutions labelled X_1 and X_2 in some cases were the same and in other cases, were different from each other. In comparing the intensity of X_1 and X_2, the subject was asked to concentrate on the flavour common in both solutions in cases where the subject perceived a combination of different flavours.

(c) Results and Discussions. The responses of the subjects to each of the ten sets of stimuli expressed as subjective judgements of comparative intensity were pooled over the twenty-two subjects. The frequency of each response category to each set of stimuli was schematically represented in fig. VI.6.

FIG. VI. 6 FREQUENCY OF EACH CATEGORY OF SUBJECTIVE JUDGEMENTS OF COMPARATIVE INTENSITY, EXPERIMENT ZINC SULPHATE MOUTHRINSE



Looking at figure VI.6, one must bear in mind that in this case the probability of each response category being chosen by the subject for each set of stimuli was p = .33. For the twenty-two subjects the expected frequency of each response category for each set of stimuli was 7.33.

It was clear from figure VI.5 that none of the sets of stimuli elicited a response category with frequency being significantly different from the expected frequency. This result indicated that none of the mouthrinses used including the zinc sulphate solutions had any significant effects on the taste sensitivity to sucrose solutions.

None of the twenty-two subjects had any difficulty in identifying the sucrose solutions as being sweet. This was because the two concentrations of sucrose solutions used were well above the normal recognition threshold concentration (Pfaffmann, 1959; Amerine, Pangborn and Roessler, 1965). Upon questioning at the end of the experiments, the subjects reported that they were in fact comparing the degree of sweetness of the pre- and post-rinse sucrose solutions during the experiments.

It could be concluded from the results of this experiment that the two concentrations of zinc sulphate solutions used as mouthrinses, did not have any significant effect on the sensitivity to sucrose solutions.

However, it cannot be over-emphasized that this experiment was only exploratory in nature. The method used was a very simple one which left much room for improvement. For further investigation in this area, it may be worthwhile to consider an application of the magnitude estimation method where the subject assigns numerical values to the intensity of flavour in both the pre-rinse and post-rinse solutions. This may allow the subject

to make more meaningful comparisons while at the same time providing the experimenter with a better idea of the subject's perception.

The choice of testing solutions whose concentrations are close to the detection threshold levels may also be of help in this situation. This will allow the detection of small changes in taste sensitivity due to the mouthrinse. An ideal situation, but not necessarily practical in an experimental setting would be to assess the recognition thresholds to the chemicals used as testing solutions for each subject, then to choose the concentrations close to those thresholds as testing solutions for that subject. However, this would make a cumbersome and laborious task for the experimenter.

Further experimentation with aims to establish the effects of zinc sulphate mouthrinses on taste sensitivity to other chemicals, of different taste qualities, needs to be carried out before any definite conclusion can be drawn concerning the effects of zinc sulphate mouthrinses on taste sensitivity.

V. SUMMARY

To summarise, in this series of experiments it was found that

(1) zinc sulphate solutions had complex taste sensations consisting mainly of bitter, sour, acid with a sensation of dryness of the oral cavity and underlying tastes of sweet and salt, together with a prickly sensation in the oral region. The intensity of these taste sensations increased as the concentration of zinc sulphate progressively increased. At very high concentrations (8 gm/lt and higher) the sensation of dryness

dominated all other taste components.

(2) When subjects were asked to taste mixture solutions of zinc sulphate and sucrose at various concentrations, the subjects responded in a manner which indicated that they could respond to each of the two components of the mixture separately. The intensity perceived of each taste component was proportional to the physical concentration of that component.

(3) In a preliminary investigation, zinc sulphate mouthrinses did not have any significant effects on taste sensitivity to the sweetness of sucrose solutions.

CHAPTER VII

SUMMARY AND CONCLUSIONS

The main aim of this research was to investigate the relationships between zinc metabolism and gustatory sensitivity which were suggested by Henkin and his associates (Schechter et al. 1972). The rationale behind the hypothesis that zinc plays an important role in taste processes has been discussed in detail in Chapter IV. At first, zinc was administered to patients undergoing treatment with penicillamine because of its lowest toxicity among the transition elements. At that stage, zinc as well as other transition elements were considered effective in the treatment of taste abnormalities because of their interactions with thiols. When zinc was first found to be effective in the treatment of idiopathic hypogeusia (Schechter et al. 1972), a complex metabolic net involving zinc and copper was suggested to play a role in taste processes. Copper was later eliminated from the consideration when changes in zinc metabolism alone were found to cause hypogeusia. At that time, McConnell and Henkin (1974) postulated that zinc played a role in taste processes through its protein synthesising activity. Changes in zinc metabolism were considered to effect taste processes through the effects of zinc on taste bud metabolism. More recently, Henkin was said to have proposed another hypothesis, namely that there is a zinc-containing protein in the normal saliva which plays a role in the growth and nutrition of the taste buds (Culliton, 1975). Henkin et al. (1975) in fact have reported successful isolation of such a protein which they called "gustin". The exact role of this protein in taste processes is yet to be established.

Let us at this point, take a look at a summary of the results of this research before further discussions.

I. SUMMARY OF RESULTS

It was not possible in this research to employ a control group of healthy subjects who would be willing to give blood samples for the analysis of zinc serum and plasma levels. Therefore, a normal value of 110 µg/100 ml as suggested by Hallbook and Lanner (1972) was used as the normal serum and plasma zinc levels. Among the alcoholic subjects studied in this research, the following results were found.

(1) Alcoholic subjects as a whole, at the time of admission to the hospital had lower than normal plasma zinc levels (mean = 99.2 $\pm$ 14.8 µg/100 ml).

(2) Alcoholic subjects who reported some subjective decrease in taste sensitivity had lower than normal plasma zinc levels (experimental group mean 93.9 $\pm$ 12.6 µg/100 ml, control group mean 92.7 $\pm$ 11.9 µg/100 ml). The plasma zinc levels of these alcoholic subjects did not change significantly during the first three weeks of hospitalization without zinc therapy.

(3) Those alcoholic subjects who underwent zinc sulphate therapy at the dosage of 150 mg Zn^{++} per day, had their plasma zinc levels raised above normal levels during the two weeks of therapy. This was supported by the findings among chronic leg ulcers patients.

(4) Alcoholic subjects who took part in the studies showed both a subjective and an objective depression in sensitivity to sweet, salt, sour and bitter stimuli. Their taste sensitivities did not change significantly during the first

two or three weeks of their hospitalization without therapy.

(5) Alcoholic subjects who underwent treatment either with placebo or oral zinc sulphate medication showed significant improvement in taste sensitivity to sweet, salt and bitter but not to sour stimuli during the three weeks of therapy. There was no significant difference in taste responses between subjects treated with zinc sulphate and those treated with placebo.

(6) Zinc sulphate solutions used as mouthrinses did not have significant effect on the perception of sweetness of sucrose solutions.

(7) Solutions of zinc sulphate in deionized water were found to have complex taste sensations consisting mainly of bitter, sour, acid with a sensation of dryness of the oral cavity and underlying tastes of sweet and salt together with a prickly sensation in the oral region. The intensity of these taste sensations increased as the concentration of zinc sulphate progressively increased. At high concentrations the sensation of dryness dominated all other taste components.

(8) When zinc sulphate solutions and sucrose solutions were tasted as mixtures, the subjects were able to judge the intensity of the component solution separately. The perceived intensity of each taste component was proportional to the physical concentration of that taste component. This finding indicated that zinc sulphate did not have significant effect on the perception of sweetness of sucrose in mixture solutions.

II. CONCLUSIONS AND DISCUSSIONS

The overall result of this research failed to support the hypothesis that zinc metabolism plays an important role in taste

processes. The finding that there were no significant differences between the responses to taste stimuli of subjects in the zinc treatment and placebo groups is contrary to the findings of Schechter et al. (1972).

As suggested earlier, there are major differences between the experiment conducted by Schechter et al. (1972) and the present study. Firstly the subjects of this study were alcoholic patients admitted to the hospital for the treatment of alcoholism who complained of subjective decrease in taste sensitivity. These patients did not seek help to improve their taste sensitivity and were not aware of the possible effect of the medication they were receiving. In the experiment of Schechter et al. (1972), the patients were idiopathic hypogeusia patients who actively sought treatment for their complaints and expected to be cured by the medication given to them.

Secondly, the present study used the sip method of tasting instead of the drop method used by Henkin and his associates. The bias problems associated with the drop method has been fully discussed.

Even though the results of this series of experiments fail to support the hypothesis advanced by Henkin and his associates, no further comparison can be made between the results of this research and those reported by Henkin and his associates due to the lack of procedural detail in the reports of Henkin and his associates, discussed earlier.

More definite conclusions could be drawn if an experiment could be conducted wherein patients with idiopathic hypogeusia were studied in a double-blind experiment using the sip method of tasting rather than the three stimulus drop technique employed by Henkin and his colleagues. The impracticality of conducting a

double-blind study in this research has been previously discussed.

Henkin in fact has conducted a double-blind study, the results of which have not been published. In personal correspondence with Gregson (1974), he said,

"The double blind study is completed but it was carried out from a quite different point of view; i.e., any patient with a taste defect from whatever cause was treated with zinc ion. It doesn't quite work that way but subgroup analysis demonstrates that some patients do benefit from zinc therapy."

Another source of information on this double-blind study comes from Culliton (1975) who said,

"Henkin conducted a double blind study. That study gave no evidence that zinc is any better than placebo in treating taste loss. To this day, the results of this study have not been published."

It is unfortunate that the details of this study has not been published. This prevents further conclusions to be drawn concerning the role of zinc metabolism and taste sensitivity.

There seems to be little future in further experiments with zinc in the area of taste functioning. This suggestion has been put to Henkin who, according to Culliton (1975), responded that,

"We now know that zinc is helpful only to those patients who are zinc depleted. Among those patients in whom we can demonstrate zinc depletion, there is a correlation with successful zinc therapy."

However, as mentioned earlier, this area of research is a very difficult one. Taste is partially a subjective phenomenon which lends itself to bias problems if the method used to measure it is not well controlled. The detection threshold, widely used by Henkin and his associates to represent taste sensitivity is particularly subjected to the problem of false positives if the testing method is weak as in the case of drop technique. The measurement of absolute thresholds, in particular the detection thresholds, had been avoided in this series of experiments,

partly because of this problem.

The clinical application of psychophysical investigation itself has many inherent problems. The measurements used in this situation has to allow for flexibilities due to the conditions of the patients who take part as subjects. The experimental conditions cannot be as rigidly controlled as in a laboratory situation. Because of these factors the reliability of the results, in certain cases, is not as high as to be expected. This also reduces the replicability of the results as apparently experienced by Henkin and his co-workers.

As discussed in Chapter II of this thesis, the knowledge of zinc metabolism is still very limited at this stage. We know that zinc metabolism is affected by many factors. Even though symptoms resulting from zinc depletion or deficiency have been noted, no universally accepted norm of normal zinc metabolism has been established. The accurate tools used in the diagnosis of zinc depletion or deficiency are still to be found. The use of plasma or zinc serum levels, though widely used, as indications of zinc supply and availability in the body is still debatable.

Therefore, before any definite conclusions can be drawn on the relationship between zinc metabolism and gustatory sensitivity, a greater understanding of zinc metabolism must be obtained. Once that point is reached, a carefully conducted double-blind study with well controlled technique of taste testing should be carried out.

As it stands, the results of this research indicate that among subjects who experienced minor decrease in taste sensitivity (in comparison to severe disturbances in the cases of idiopathic hypogeusia), zinc does not have a significant role in taste processes. In patients who exhibited low levels of plasma zinc,

zinc medication, while raising the plasma zinc levels above normal values, did not have a significantly different effect on taste sensitivity from the effects of a placebo.

It is, of course, possible that the role of suggestibility factors in affecting taste detection responses is enhanced in patients with syndromes such as alcoholism. There exists a wide but inconclusive literature on personality anomalies in alcoholics, but no literature on the psychological aspects of idiopathic hypogeusia. If this latter syndrome, identified by Henkin and his colleagues, does involve marked psychosomatic components, reminiscent of taste and smell aberrations in hysterias, then exaggerated responses to any noisy detection task could be expected. It is not impossible that Henkin, like many other physicians before him, working on neurological sensory disorders, has been misled into seeking physiological and biochemical explanations for cognitive and perceptual maladaptive behaviour patterns.

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APPENDIX I

QUESTIONNAIRE FORM

USED IN

EXPERIMENT ALCOHOLIC TASTE II

AND

EXPERIMENT ZINC SULPHATE TREATMENT AND TASTE

NAME: SUBJECT NO.:

AGE:

SEX:

DATE ADMITTED:

DATES TESTED - I

II

III

IV

MEDICATION:

DENTURES WORN:

SMOKING HABITS:

HISTORY AND MEDICAL EXAMINATION RESULTS:

APPENDIX II

RECORD SHEET OF SCALES USED IN EXPERIMENT ZINC SULPHATE TASTE I EXPERIMENT ZINC SULPHATE TASTE II AND EXPERIMENT ZINC SULPHATE TASTE III

SAMPLE NO. _____ SUBJECT NO. _____

TASTE / RATING	SWEET	SALT	SOUR	BITTER	ACID	DRY	PRICKLY	WATER
EXTREMELY STRONG								
VERY STRONG								
STRONG								
FAIRLY STRONG								
INTERMEDIATE								
WEAK								
VERY WEAK								
FAINT								
JUST DETECTABLE								
UNCERTAIN								
NOT DETECTABLE								